

A C O N T R I B U T I O N

TO OUR KNOWLEDGE OF THE SPECIFIC

GRANULES IN THE CYTOPLASM OF LEUCOCYTES

WITH SPECIAL REFERENCE TO THE ACTION OF

NUCLEIC ACID UPON THEM.

(A STUDY IN THE GENERAL AND INTRACELLULAR
METABOLISM OF THE NUCLEINS).

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JOHN MALCOLM, M.B. Ch.B. (1897)



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Before taking up the study of the granules found in leucocytes, it would be as well to consider shortly the structure and chemistry of animal cells in general.

Morphologically, a cell is found to consist of a mass of apparently structureless material enclosing a definite, more or less rounded body- the nucleus. These two parts- cell body and nucleus-are so distinct, structurally, chemically and physiologically that they must be regarded as differentiations of the general protoplasm of primary importance. Another body present in almost all cells has been much emphasised lately as a permanent cell organ viz., the centrosome.

The cell body is bounded by a membrane in very many cells; this either arises as a secretion from the cell or consists of the condensed outer layer of the cell protoplasm.

The structure of protoplasm has been the subject of frequent investigation and is still to a great extent unknown. Under the highest powers of magnification at present available, the appearances seen depend to some extent on the mode of treatment e.g., granular appearances after fixation with platinic

platinic chloride- but, discounting the effects of reagents, most workers agree that it is of the nature of a network- real or apparent.

Three chief theories are held as ^{to} the interpretation of this.

(1) Arnold,¹ Flemming² and others hold that there is a real fibrillar, sponge-like structure composed of what they call linin- the interstices being filled with a clear fluid called hyaloplasm.

(2) Bütschli³ and his followers, working at the subject from the appearances seen in sections of artificial emulsions have come to the conclusion that the fibrils of the network are optical sections of alveolar walls of a dense substance enclosing a less dense- giving rise to a "foam-like" structure.

(3) Altmann⁴ holds that protoplasm consists of a clear homogeneous basis in which are embedded fine granules (microsomes) which are arranged in linear series to form fibrils.

Whichever view of the netlike appearance is taken, it is tolerably certain that the network of the nucleus is continuous with that of the general cytoplasm, for besides the fact of both being traceable up to the nuclear membrane, Wilson⁵ has shown

shown that in those cases where the centrosome divides and forms the spindle inside the nucleus by a modification of the nuclear reticulum, the half of the spindle found in each daughter-cell goes to form part of the cytoplasmic reticulum of that cell.

Granted then this all pervading structure, be it fibrillar or foam-like, we have next to consider shortly the permanent cell organs- nucleus and centrosome. These undoubtedly arise by certain local differentiations of the protoplasm due in the end to chemical activities of different degrees and nature. Thus the nucleus becomes marked out as the area where certain metabolic changes take place accompanied by the presence of nuclein insufficiently saturated with albumen (= "chromatin") ; the centrosome in the same way must be an area with a specific chemical activity capable of inducing the phenomena of cell division.

In all questions of cell physiology the nucleus is of prime importance- as it has been frequently shown to be the trophic centre of the cell, and the chief object of the present paper is to show the intimate connection between it and the cytoplasm with regard to the metabolic changes in one great type of

of cell viz., the leucocyte.

The nucleus is usually a definite rounded body surrounded by a membrane and composed of a reticulum formed by masses of chromatin embedded in a clear fibrillar (or alveolar-like) substance.

During cell division these masses form a definite number of rod-shaped bodies in which the chromatin is present in a granular form- and some observers have described the chromatin of "resting" nuclei as composed of the same ultimate structure.

Heidenhain⁶ has described other granules present in the nucleus, which he terms oxychromatic or lanthanin granules- these stain with acid dyes, and he states that they may become true chromatic granules. This is a point in favour of Altmann's theory of the cell being composed primarily of lesser units (microsomes.) These oxychromatic granules have recently attracted much attention from histologists.

As to the idea that they are fore-runners of the chromatin granules, one must remember that the products of the regressive metabolism of the chromatin will probably leave it in granular form, so that the above mentioned granules may quite as well arise from the chromatin as go to form it.

it.

The nucleoli found in many nuclei fall under two categories- (1) nodes of the chromatic network, and (2) a mass of substance called pyrenin which stains with acid dyes. The extrusion of nucleoli from the nucleus into the protoplasm of the cell has been studied by many observers- in this country especially by Dr Carlier⁷ of this University. I shall speak of cell granules later on.

In studying the structure of cells, histologists ~~had~~ ^{have} long been in the habit of using certain dyes which differentiated the cell organs, but their mode of action has only recently been to a small extent elucidated.

Ehrlich⁸ classified the dyes according to their acid or basic properties as opposed to mere colour effects. Lilienfeld⁹ showed that nucleic acid, which Miescher had found to be the most important constituent of nuclei formed variously coloured precipitates with methyl green according as the nucleic acid was more or less saturated with albumen.

Mathews¹⁰ has advanced our knowledge in this direction by showing that the acid stains form a true chemical combination with albumins and

and albuminoses. This only takes place with coagulated albumins if the solution of the stain be acidulated, so as to set free the coloured organic acid in a nascent state. He considers that in such cases it enters one of the basic N H_2 groups of the albumin molecule.

On the other hand, basic stains unite with albumin when in alkaline solution. Here he considers that the stain enters the hydroxyl of the phenol group of the molecule.

Strong organic acids, such as nucleic acid, have a greater affinity for the basic stains than for the albumin which is usually bound to them. Albumen itself acts as an acid but as such is so weak relatively to nucleic acid that it acts the part of a base to the latter.

The basic stains are not tests for chromatic matter alone, but indicate the presence of any strong organic acid such as the above or the chondroitin-sulphuric acid of hyaline cartilage. In the nuclei of cells, however, the only organic acid of any strength which has been separated is nucleic acid.

Among other microchemical reactions may be mentioned those for glycogen, fat- iron and phosphorus.

phosphorus. This last is very interesting from the amount of work that has been done on it and from its great importance. Unfortunately, in spite of the labours of Lilienfeld and Monti,¹¹ Heine,¹² and most recently Macallum-¹³ the methods are still very crude.

Chemistry of the Cell.

Our knowledge of the chemistry of the cell is largely based on work done on leucocytes and their derivatives.

The most interesting and distinctive constituent of simple cells such as leucocytes is nucleic acid discovered by Miescher¹⁴ in 1874 and worked at by Altmann,¹⁵ Kossel and others.

It is a fairly stable body, united in the nucleus to albumin to form "nuclein" and this again has still more albumen attached forming the "chromatin" of histologists.

In leucocytes the nucleic acid is also united to albumen forming a nuclein. To this an albumose-like body- histon- is attached.

Thus the following series can be differentiated

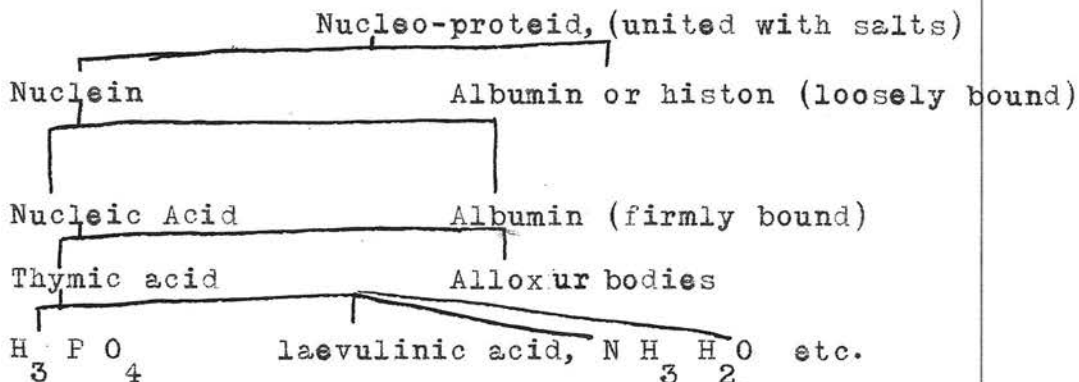
- (1) Nucleic acid^{free,} containing about 10% Phosphorus
- (2) Nucleic acid + firmly bound albumin = nuclein (5% Phos)

- (3) Nuclein + Histon = nucleo-histon found in leucocytes
 (4) Nuclein + loosely bound albumen = nucleo-proteid
 (5) *Nucleic acid + Protamin = Nuclein of Spermatozoa heads.*
 On hydrolysis with weak acids nucleic acid breaks

up into certain bases containing the alloxan nucleus (xanthin, adenin, hypoxanthin, guanin, etc.) and an organic acid containing all the phosphorus of the original nucleic acid termed thymic acid.

On further action this, in its turn, breaks up yielding phosphorus as $H_3 P O_4$ and its nitrogenous part as $N H_3$ etc. As I have to deal largely with the changes which those bodies undergo in the cell it will be as well here to give a short scheme of the way in which a nucleo-proteid breaks down and also indicate its possible building up.

Disintegration.



Reintegration.

The nuclein derived from food, on digestion gives peptone and an organic phosphorus-holding acid united

united to albumose. In the mucous membrane the latter forms albumin ~~again~~ and the former most probably forms a nuclein again and reaches the tissue cells as such.

As it has been my aim throughout this research to shed some light on the interchange which takes place between nucleus and cytoplasm in the leucocytes, it is essential to have a clear knowledge of the decomposition products of the nucleins.

It will be seen that there are two important decomposition products of nucleins which might be gauges of the amount broken down- viz., $H_3P_4O_{10}$ and the alloxur bodies (uric acid and nuclein bases.) Each of the bases (xanthin etc.,) is supposed by some to form part of a special nucleic acid so that there would be four such at anyrate, but as yet no single form of nucleic acid has been isolated which yields only one of these bases on decomposition.

As already said, nucleic acid unites with albumin in varying proportions which give rise to differences in reaction to basic stains but in the heads of spermatozoa nucleic acid is united to a strongly basic body "protamin" which is the simplest proteid at present known. This causes the heads of the spermatozoa, though so very rich in nucleic acid, to react to certain stains, (e.g., eosin) as if it were acidophil.

Another important constituent of the cell is a body closely allied to nucleic acid viz., paranucleic acid-

acid- which unites with albumin to form a paranuclein, and this again with more albumin to form a nucleo-albumin- the corresponding compound of nuclein and albumin being nucleo-proteid.

It is in all probability very closely allied to thymic acid- it yields no alloxur bodies on decomposition.

The relation of this body to true nuclein is an intimate one, as shown in the secretion of milk. Here the nuclei of the mammary gland cells yield their true nuclein which is split up to form a paranuclein united to albumin (caseinogen) and alloxur bases which most probably return to the nucleus to help to form more nuclein.

Other constituents of simple cells are albumins, globulins, lecithin, salts, etc.

These do not seem to be so intimately bound up with the life of the cell as is the case with the organic phosphorus-holding acids above mentioned.

In order to study the relationship which exists between the morphological and chemical constitution of cells and between the various parts of the cell, one must use methods which will, by

by altering the constituents of the nucleus and cytoplasm respectively, allow one to find out in what ways the general cytoplasm may be affected by the nucleus.

One must therefore take up a group of cells, the life-history of which can be more or less easily affected by physiological or pathological conditions, and hence the choice of the leucocytes is a natural one.

Before discussing the intimate changes which the leucocytes undergo in different conditions, it would be as well to find out how the general metabolism is affected by a mere increase in their numbers in the circulating blood.

This leads me to speak in the first place of the different views at present held, as to the cause and nature of this increase.

Leucocytosis in general.

One of the earliest views on this subject was that of ¹⁶~~Von~~ Limbeck, who held that the increase was due to a concentration of the blood from exudation, but this theory is untenable since one may have a marked hyperleucocytosis with no trace of exudation.

¹⁷
Römer's view based on Buchner's and his own

own work was that hyperleucocytosis arises from decomposition products obtained from the bodies of dead bacilli or other cells- these acting as ^{direct} formative irritants on the white cells ^{in the blood.} ~~directly~~. He does not believe that the leucocytes present in the blood in hyperleucocytosis are already present in the blood-forming organs.

Against this there is to be urged the absence of *Signs* of division in the blood in ordinary leucocytosis and also the evidence of activity of blood forming organs.

¹⁵ Löwit stated that hyperleucocytosis was usually preceded by, and was dependent on, a hypoleucocytosis. He mentions two types of hypoleucocytosis viz., - (1) leucopenia due to cold or shock from a diminution in numbers transferred from the blood formative organs, along with slight breaking down and (2) leucolysis due to destruction of leucocytes after injection of different substances e.g., bacterial products, albumoses, nucleic acid, etc. In all these cases he found a diminution followed by an increase of numbers which was proportional to this diminution.

This theory has been proved fallacious by Goldscheider and Jakob ¹⁹ who found that they could get

get a hyperleucocytosis experimentally by repeated small injections of leucocytotic agent without any preceding hypo-leucocytosis.

Schultze²⁰ holds that there is no such thing as a hypo- and hyper-leucocytosis but that it is merely altered distribution of cells from skin capillaries to internal organs. It has however been found by many observers that though there is always a greater number of leucocytes in blood got from the skin than from a deep vein, yet the same holds good throughout the variations of numbers occurring in hypo- and hyper-^lleucocytosis.

The theory of chemotaxis advocated by Goldscheider and Jakob is one of very great interest.

Their researches were carried out on rabbits and the results refer to number and distribution of leucocytes after subcutaneous and intravenous injections of various organic extracts.

They agree with Löwit as to the occurrence of a preliminary hypo-leucocytosis- thus 2 c.c. of bone marrow injected subcutaneously into a rabbit caused a hypo-leucocytosis- the numbers falling from 8,200 (per cmm) before to 4,000 three hours after. In six hours the blood count was 18,200, and this gradually

gradually ^{tell to} ~~reached~~ normal in 36 to 48 hours. Splenic extract, thymus extract ⁺ hemialbumose, also caused similar results, the hypo-leucocytosis appearing in 3 to 4 hours. Nucleic acid .2 to .5 gm. was also used ^{resultant} the hyper-leucocytosis being moderate ~~after it~~.

To find the reason of this diminution they went on to examine the organs of animals killed in that stage, arguing from the point of view that when the injected substance entered the venous system the capillaries of the lungs would be a good place to study the resultant diminution, but instead of this they found in almost every case a heaping up of leucocytes in the lung capillaries. These were of all types but more especially the polymorphic ~~leucocytes~~.

In animals which died from ether narcosis or were killed after injection of renal or pancreatic extract no such ^{accumulation} ~~heaping up~~ was found. In the stage of restitution from hypo-leucocytosis to normal (in cases where hemialbumose had been injected) they found ^{a greater accumulation} ~~more heaping up~~ of the leucocytes ^{in the pulmonary capillaries} than in the true hypo-stage. In the stage of hyper-leucocytosis the accumulation of leucocytes in the lung was found to be even still more marked- liver and kidneys also showed some increase but not nearly so great.

great.

At a later period when the number in the blood had reached normal, the capillaries in the lungs contained corpuscles which showed signs of breaking down as seen in the irregular outline of cells and isolated masses of chromatic matter. At the same time there was an increase of leucocytes in the capillaries of the liver.

By injecting more of the leucocytotic agent during the stage of hypoleucocytosis they found that if the minimum had not been reached the stage was prolonged, but if the minimum had passed, a hyperleucocytosis occurred followed by a second hypoleucocytosis and a ^{succeeding} ~~second~~ hyperleucocytosis.

A re-injection during the stage of hyperleucocytosis was followed by a slight hypo-stage, but a very marked hypoleucocytosis occurs if the substance injected the second time ^{be} ~~is~~ a different leucocytotic agent from the first one.

As mentioned before they were able to prevent the hypoleucocytosis stage altogether by injecting small amounts of the agent frequently. Such injections resulted in a very marked hyperleucocytosis.

On these facts Goldscheider and Jakob built up

up their chemotactic theory to explain the phenomena. They consider that the hypoleucocytosis is caused by a driving of the leucocytes into the capillaries of the lungs from negative chemotaxis. The subsequent hyperleucocytosis is not due to a redistribution of these cells but (1) to an increased transportation of leucocytes from the lymph canals into the blood stream due to the presence of the injected agent, and (2) to a withdrawal of leucocytes from their formative centres in bone marrow.

The causes of these two phenomena- hypo- and hyper-leucocytosis may continue to act at the same time- that is, the driving of the leucocytes into the lung capillaries may continue while they are being withdrawn from the lymph channels and formative centres.

They refer the hypoleucocytoses of some severe infectious diseases to the same kind of action, that is, negative chemotaxis, though in some cases it may be due to paralysis of the amœboid activity and proliferative power of the leucocyte.

In their work as above given, Goldscheider and Jakob studied merely the numbers and distribution of the leucocytes without attending to any special variety

variety. From the comparative rarity of increase of lymphocytes alone the term "leucocytosis" has generally come to be accepted as increase in the polymorphonuclear, finely granular leucocyte- but there is another type of cell of great importance (which may undergo an increase) and which I wish specially to refer to viz., the eosinophil leucocyte.

Long before the work of Goldscheider and Jakob, Ehrlich had in 1879 classified the leucocytes according to the reaction of the granules which they bore when fixed by heat. He drew special attention to the coarse eosinophil cells as being the most highly specialized of all granular cells and pointed out that in each species of animal the eosinophil cell had some special characteristic: e.g., the large pale granules in the horse, the spindle shaped crystalline rods in birds, etc.

Ehrlich found that in cases of acute general leucocytosis the coarse eosinophil cells either did not share in the increase or were diminished, while in leucocythæmia there was usually a great increase.

The value of this mere increase in numbers in leucocythæmia fell into discredit when numerous observers began to find an increase in other diseases.

diseases.

Müller and Rieder²¹ in 1891 and Weiss²² also in same year emphasised the form and character of the eosinophils in leucocythæmia (as opposed to numerical increase) and in this country the same points were observed by Muir.^{22a}

There was found to be an increase in eosinophilous cells in many diseased conditions which seemed to have no common binding link, hence there was at first considerable enthusiasm over Neusser's²³ statement in 1892 that such a link existed in the sympathetic nerve. He stated that in all diseases accompanied by eosinophilia there was at the same time a disorder of the sympathetic nervous system. Among diseases coming under this category he mentions osteomalacia, ovarian and sexual disturbances, sarcoma, pemphigus, pellagra, Basedow's disease, myxœdema, hysteria, and the train of symptoms classified under the "uric acid diathesis." All these, he believes, are primarily due to a stimulation of the sympathetic and that eosinophilia is a constant accompaniment of such stimulation.

This theory of Neusser is not accepted by many observers mainly on the grounds that eosinophilia

eosinophilia may occur in cases that present no symptom of any disease at all and it loses a great part of its value when we remember that the range in healthy individuals is so great (.67% to 11 - Zappert) that an estimation of their number is generally only of value when we know the number normally present in that case.

Zappert²⁴ in 1893 gives a very good account of the numbers of eosinophils in many diseased conditions. Speaking generally, a diminution of ~~eosinophiles~~ ^{these cells} was found during the height of most febrile conditions. After the fever has passed, they increase and may rise above normal. That the increase is not due to mere rise of temperature is shown by the fact that they may be increased in some cases of fever, e.g., scarlatina and malaria.

In pneumonia the reaction of the eosinophils is very interesting. Zappert, Türk²⁵ and other observers agree that there is a diminution during the greater part of the fever- the cells may even disappear while a leucocytosis as a whole is present. At twenty-four to forty-eight hours before the crisis Türk found the beginnings of an increase in their numbers or a reappearance. Zappert says they do not increase

increase till soon after the crisis but the greater importance is perhaps to be attached to Türck's work.

The reappearance of the eosinophil cells signifies that the infection has passed its height, and taken in conjunction with other signs means that the prognosis is a favourable one.

In pulmonary phthisis with or without marked febrile attacks there is, according to Zappert, a distinct diminution of eosinophil cells and in cases ^{after} ~~of~~ tuberculin injection there is a marked diminution during the febrile stage. With repeated injections there is a marked increase shown, the numbers rising to even three times the usual percentage. On stopping the injections the numbers fall to normal again.

Scarlet fever frequently shows an increase, while septic infection usually causes a diminution.

Looking at eosinophilia as a whole we see that as yet it is to a great extent unexplained, but there seems to be no doubt ~~but~~ that they are cells of vast importance in the successful reaction of the tissues to bacterial invasion and one might appropriately refer here to Kanthack and Hardy's well known work on the respective functions of the coarsely granular oxyphil cells and the hyaline cells.

cells.

The most important recent contributions to our knowledge of leucocytosis is the work of Professor Muir of Glasgow, an outline of which was given at the last annual meeting of the British Medical Association.

The experiments were done on rabbits and consisted in the production of leucocytosis by intra-peritoneal injection of staphylococci.

The marrow of such animals showed hyperplasia of the large finely granular myelocyte- diminution of eosinophil cells, relatively at any rate.

The erythroblasts and giant cells were also diminished. The convenient term "leucoblastic" type of marrow is used in contradistinction to the "erythroblastic" type seen after hæmorrhage.

These changes allow him to formulate the very probable theory that local leucocytosis is set up by chemotactic action of local bacterial invasion. If this focus is extensive enough the absorption of the diseased products causes a withdrawal of the fully formed leucocytes from the marrow into the blood- then follows a stimulation of the leucoblasts to active proliferation and thus the supply of leucocytes is kept up in the blood.

blood.

Having traced thus briefly the various views on leucocytosis as far as at present published, I have now to take up the alterations in general metabolism produced by an increase in their numbers in the blood.

As mentioned previously, Kossel and others found that nucleic acid underwent artificially a series of decomposition changes which resulted in the formation of nuclein bases and phosphoric acid. Following up this indication, Horbaczewski²⁸ made an interesting series of experiments on the bodies derivable from nuclear rich tissues when placed under the influence of simple oxidising agents. For this purpose fresh spleens were placed in defibrinated blood and allowed to "digest" at 40° C for varying periods. He found, that, while fresh spleen yielded on analysis practically no uric acid and no nuclein bases, yet a spleen treated as above could be made to give either uric acid or the nuclein bases according as the access of oxygen to it were free or limited respectively.

Proceeding still further, on the idea that a condition such as leucocytosis should indicate an

an increased rate of nuclein katabolism, Horbaczewski went on to examine the urine of such cases and stated that he found an increase in the alloxuric nitrogen (uric acid and nuclein bases) with every increase in number of leucocytes.

These observations were repeated by many observers, all kinds of leucocytosis being used for the purpose quite irrespective of the cause and specific action of the substances used experimentally. As I shall show presently these observations are unfortunately not very reliable.

During the winter session 1897-98 in conjunction with Dr T.H. Milroy of this university I conducted a research²⁹ into the subject of nuclein metabolism^{in man}, the main results of which were embodied in a paper published in the Journal of Physiology ~~for the~~ July ¹⁸⁹⁸ following.

The question we set ourselves to solve was, what are the alterations in general metabolism following the introduction of nuclein and its products into the system.

From Dr Milroy's previous work on the nucleins³⁰ we knew that the phosphorous-holding part of the nuclein was absorbed ~~being~~^{while} still in organic combination. The introduction of this body into the blood had been found

found to produce a distinct leucocytosis and the question which now arose was, whether this was simply a chemotactic effect with redistribution of the cells when the substance had disappeared from the blood or *whether* ² ¹ was there also an altered metabolism or even destruction of the nuclein of the leucocytes.

If the former were the case, the surplus products of nuclein excreted would be simply equivalent to that derivable from the absorbed nuclein:- if the latter, there would be an increase above this amount.

As a gauge of the nuclein metabolism we used the P_2O_5 excretion in the urine- (see scheme of decomposition of nuclein). This was examined not only in its absolute amounts but also in the amount of P_2O_5 relatively to the nitrogen pointing to the decomposition of some phosphorus-rich body. The advantage of this method of analysis is very apparent- since the methods for estimating the nuclein bases are by no means so exact as that for phosphorus, and moreover, it is quite possible that a varying proportion of the nuclein bases derived from nuclein in the body may be transformed into urea before excretion- a fallacy which cannot occur in the case of phosphorus.

phosphorus.

We examined the nucleins in following way- first, nuclein in the form of small doses of thymus tabloids was given and the effect on the excretion of total nitrogen (Kjeldahl process), P_2O_5 (Kjeldahl-³¹ Wiebull method), uric acid (Salkowski-Ludwig method) and nuclein bases (Camerer's method in the earlier analyses, Salkowski's ^{more} ~~new~~ recently published method in the later³²).

For the technique of those methods see paper.

Then nucleic acid itself was taken in the same way and then mono-metaphosphoric acid. The results with nuclein and nucleic acid were a distinct increase in the P_2O_5 excretion far above that derivable from the phosphorus taken. This was accompanied by a leucocytosis of moderate amount, 10,000 to 11,000 per cmm. There was little or no appreciable effect on the uric acid and nuclein base excretion. *The monometaphosphoric acid had no effect either on the P_2O_5 excretion or the number of leucocytes.*

Given, then, this increased P_2O_5 excretion, the next question which arose was as to how this was derived from the leucocytes.

For example in the leucocytosis which we got was there really an increased leucolysis?

From Goldscheider and Jakob's work it seems

seems more likely to be due to an altered metabolism of the cell seeing that most of the changes with regard to numbers of leucocytes can be accounted for from the standpoint of chemotaxis.

There is no definite sign of an increased leucolysis even in the earlier stages (hypoleucocytosis) such as Löwit imagined. If therefore there are no signs in the circulating blood of well marked morphological alterations in the leucocytes ^{in man} one is driven to the examination of the seats of formation of these cells ^{in animals,} in order to find out what changes they undergo at places where they are present in large numbers. *It was also advisable to investigate the action of larger doses than could be possibly tolerated in man.*

The leucocytes which are undoubtedly of greatest importance in the nucleic acid leucocytosis are the granular ones (fine and coarse) and it did not seem too much to expect that in the bone marrow the mother cells of those might show alterations in their number and general character.

For this purpose I had recourse to experiments on animals and as it was difficult at first to know how or what special animals would be most suitable for showing minute changes in the leucocytes, I had to try various species. The animals experimented on were

were hens, guinea-pigs, dogs and rabbits, and as it is on the last mentioned that I got the most appreciable effect I shall describe those experiments more fully. Before, however going on to speak of the changes in any one class of animal I shall here shortly refer to the nature of the granules that are to be met with in different classes ~~of animals~~ and more especially of the oxyphil granules (*fine and coarse*).

On the Oxyphilous Granules of Leucocytes.

In 1878-1880 the granules previously observed in leucocytes by Wharton Jones,³³ Schultze and Rindfleisch,³⁴ Ranvier^{34 a} and others, acquired, ^{owing to} by Ehrlich's discovery of their staining properties and their diagnostic significance, an importance which perhaps had never before been attached to any one type of cell *in the blood*.

Although much of that importance was at the time overestimated there remains to us much that is instructive and of practical utility in the study of the eosinophil cell.

The granular leucocyte has a wide distribution but it is with those found in mammals that I intend dealing in the present paper.

The term "eosinophil" was originally used by Ehrlich

Ehrlich and is of classical interest in this respect. It has been modified by later writers to "oxyphil" (Kanthack and Hardy), and "acidophil"³⁵ (Gulland). These latter terms are preferable as indicating the more exact nature of the affinity.

The coarse oxyphil granules are easily seen in unstained preparations, such as simply dried blood films:- they are round or oval ~~in~~ highly refractile bodies filling up the whole cell body. Their size in each cell is fairly uniform when the cell is intact; but when the cell is broken down so that one sees more of the interior some differences in size may be detected, the smaller ones being nearer the nucleus.

The cells bearing those granules have a varied distribution being present in all blood channels and spaces and also to a variable extent in lymph spaces, serous sacs and connective tissue spaces. Hence Kanthack and Hardy have attempted to classify the cells into haemal and coelomic groups but the interchange between the two must be so free, as Gulland says, that such classification is not of great practical value.

The affinity for acid dyes which they all possess varies to a considerable extent both between

between the fine and coarse granules of any one animal and between those of different animals. We are indebted to a method introduced by Kanthack and Hardy, based on the work of Ehrlich and Schwarze for the detection of differences in degree of this affinity.

It depends on the fact that acid dyes such as eosin possess less and less staining power according as one uses the dye in watery-glycerine or alcoholic solution and that various degrees of this can be brought out by using eosin in alcohols of varying water percentages- the power of staining increasing directly as the water percentage. It is as well perhaps to remark here that heat fixation raises the acidophile tendency of the granules, as stated by Ehrlich and many others.

The "neutrophilous" granules of Ehrlich ^{are} ~~is~~ now included under the term "oxyphil" as ^{they have} ~~it~~ has a distinct though weak affinity for strong acid dyes.

Corresponding to the neutrophilous granules found in man, Ehrlich described granules found in the rabbit and other animals which could be stained with either basic or acid dyes - amphophilous. The neutrophilous and amphophilous granules are smaller than the eosinophilous granules of the same animal but

but the cells bearing them are more numerous and generally larger. In birds the coarse eosinophil granules are of two kinds- round and spindle-shaped- the latter being frequently found with a vacuole in the centre and they are very frequently found free near the cell hence Zappert is not correct in saying that all the eosinophil granules which are free are artefacts.

Opinions as to these cell granules have chiefly been expressed with regard to the coarse eosinophil and have been mostly founded on their staining properties and resemblance when stained to substances of known chemical nature such as hæmoglobin.

The incompleteness of this method may be gathered from the earlier part of this paper where the use of stains as microchemical tests is spoken of.

Many observers down to the time of Ranvier (1875-78)³⁶ described the coarse eosinophilous cells as fatty, a view which was still maintained by Litten³⁷ as late as 1892. Ranvier described them as partly fatty and partly of some other substance stainable with carmine Pouchet³⁸(1879) advanced the theory that while some were fatty some were composed of hæmoglobin and at present many French authors consider them of the

the latter nature whilst most German authorities consider them albuminous. Ehrlich does not give any very precise opinion on their chemical nature.

Kanthack and Hardy held that they were the parts of the cell which carried the alexines.

According to all these they are paraplastic but Arnold and others, among whom is Dr Gulland, hold that they are the nodes of the cytoplasmic reticulum.

³⁹ Sakaroff thinks that they are produced by a phagocytic action on the part of the leucocyte towards the ~~extended~~^{ru} nuclei of red cells in the marrow. He says that the nucleus is absorbed by the leucocyte and that its nucleoli go~~x~~ to form the eosinophil granules.

The most recent paper is that of Bogdanoff.⁴⁰ He examined the granules found in the liver of axolotl and from their reaction with the iron-alum hæmatoxylin method he considers them as closely allied to nucleoli, and that they are of the nature of hæmoglobin but that at a later period they become fatty. He does not mention an important point mentioned by Ehrlich viz., that according to Barker⁴¹ they are iron holding; but even this fact is not conclusive to their hæmoglobin nature, since many nucleo-proteids are also iron

holding.⁴² Sherrington surmised that they might be

be nucleo proteid but on very insufficient grounds, viz., on their being held to give the phosphorus reaction. As stated previously there is no test for phosphorus at present applicable to cell granules and even if it were proved, one had still to exclude lecithin.

Now, it is clear that one cannot speak of the character of these granules from their staining affinities alone, still less can one speak of their relationship to the nuclear metabolism. It is absolutely essential to find out to what class of bodies they belong in order to discuss their source of origin. With this purpose in view I instituted the following series of experiments:- Marrow films of guinea pig were fixed with heat and subjected for various lengths of time to the following reagents:-

- i Alcohol- boiling to remove ^elecithin
- ii Ether - boiling to remove fat and ~~l~~ecithin
- iii Dilute alkalis to remove albumins or bodies of a proteid nature such as nucleo proteid
- iv Dilute acids such as acetic, oxalic, also to remove nucleo proteids
- v Sodium ethylate in alcoholic solution

In trying the effect of any reagent at high temperatures it was of course important to prevent partial loss by evaporation. Hence the film was

was placed in a specimen tube or very small beaker which was tightly corked- the cork or rubber stopper being pierced by the nozzle of an upright water condenser which was fixed above a water bath kept *at a temperature of about 100° C* ~~boiling or nearly so.~~ The tube or beaker was partially immersed. After a varying interval the film was taken out, washed and brought back to its original condition as far as possible.

i Alcohol

Here only pure neutral alcohol was used. ¹ It was changed twice at intervals of ten minutes so that the film was subjected to it for half an hour in all at its boiling point. The film was then dried in the air and stained for twenty-four hours in weak eosin (.25% in 10% alcohol) then stained for one minute in Hansen's hæmatoxylin (Stöhr.) In some cases eosin and methylene blue staining was used.

Results

Neither the fine nor coarse oxyphil granules are in the least extracted, but the coarse granules are not quite so distinct as normal. The nuclei of all the cells stain very brilliantly in the hæmatoxylin. The nuclei of the erythroblasts are seen to be ⁱⁿ speckled with areas stained with eosin; these may be

be granules but are more likely to be vacuoles in the nucleus with the cell body shining through.

ii Ether

Pure sulphuric ether was used at its boiling point in the same way. It was also used in conjunction with, and following previous extraction with alcohol for upwards of one hour.

Results

The effects were practically the same as for pure alcohol. Neither form of granule was removed but the coarse ones were apparently altered in their refractile power and their margins seem to blend to some extent. The same effects followed in the case of granules of the leucocytes in the blood of the guinea pig. The fact that the granules were certainly not extracted by boiling alcohol or ether removes the possibility of their lecithin nature and it also makes it pretty certain that lecithin is not even combined with another substance in the granule. Neither can they be of fatty nature as shown by action of boiling ether.

Thus having excluded bodies of both lecithin and fatty nature, the only other organic class to which they might belong was the proteid group.

Hence I went on to try the effect of weak alkalies and acids in different strengths in order to find out

out if possible to which group of proteid they belonged. Before speaking of the action of these solvents I must refer to a point, the importance of which is apt to be lost sight of, viz., the effect of different degrees of heat fixation and the mode of application of the heat on the chemical nature of the substance in the granule. What I specially refer to is the tendency to the formation of insoluble coagulated albumins in cases where the temperature is gradually raised in contradistinction to the rapid removal of water without true coagulation in cases where the film is suddenly plunged into a temperature already high. One would expect to find a more soluble condition of the granules in the latter case and I found experimentally that this was so- hence my further manipulations were done on films fixed at temperatures of 115°C to 130° from the outset.

iii Action of Alkalies.

Alkaline solutions were used in various ways:- as solutions in alcohol of varying water percentage used at boiling point- ^{and secondly} as watery solutions in the cold.

(a) .07% sodium hydrate in 90% alcohol was used at its boiling point for half an hour; the film

film was then washed thoroughly in alcohol- tap water- acidified water- tap water again ^{then} alcohol and dried in the air and stained as before.

Result

Here the action on the granular cells is a very marked one, many granular cells being seen with brilliant coarse granules, others present unstained areas of about the same size- these may correspond to other coarsely granular cells in the marrow such as the coarse basophil or the orangeophil-granular cells of the guinea-pig. As to the amphophilous the great majority are removed entirely.

In some cases the films seem much corroded by the action of the reagent, but what strikes one is the persistence of two structures- the nuclei and the coarse eosinophil granules.

(b) Watery alkaline solutions. Here the films were placed face downwards on $\frac{1}{2}$ to 1% solution of sodium carbonate for from one to sixteen hours, then carefully washed as above.

Results

Here also the amphophilous granules are in great majority removed whilst the eosinophil granules are left and stain much more brightly than the few amphophilous ones which are left. The haemoglobin of the red blood corpuscles is completely gone in

in this experiment as in the alcohol and soda one, the red corpuscle being now represented only by an area bounded by a thin streak which must represent the original cell membrane while inside this, one frequently sees irregular star-shaped particles. The application of the same methods to marrow films of other animals gave the same result. In rabbits especially the two forms of acidophil granule are removed almost entirely; the nuclei of the amphophilous cells are vacuolated and the groundwork of the cell presents fine vacuoles set in a pinkish basis, (specimens stained with eosin and methylene blue).

iv Action of Acids.

(a) Acetic acid was used in alcoholic solution .4% and 1% in 95% alcohol at its boiling point for half an hour. The films were then washed in tap water- water with alkali, etc., as in other cases.

Results

Here there is a partial removal of both forms of granules, those left being swollen up and stained somewhat diffusely with eosin. Some cells present unstained areas (vacuoles) corresponding in size and position to eosinophil and amphophile granules. The hæmoglobin of the red blood corpuscles is in great part preserved and takes on its characteristic colour

colour with both eosin and ~~with~~ methylene blue.

Practically the same holds good for the granules in the haemal leucocytes but they seem to be slightly more resistant.

(b) Oxalic acid. This was used because of the double action of acetic acid in removing some parts and causing others to swell up. (1) Oxalic acid in .4% in 70% alcohol kept boiling for half an hour and then treated and stained as before mentioned gives a much sharper picture.

Results

Again there is a partial removal of both forms of granule, vacuoles being seen corresponding to the removed granules. Many cells contain these vacuoles mixed with oxyphil granules. The nuclear matter is well seen but the haemoglobin of the red cells has disappeared.

(2) Oxalic acid in watery solution (1 $\frac{1}{4}$ %) for one hour at 37°C showed removal of both forms of the granules but many of the coarser vacuoles which corresponded to eosinophile cells contain minute star shaped remnants the colour reaction of which is difficult to make out.

The nuclear matter is very distinct and well stained but the nuclei are vacuolated. In rabbits the

the amphophilous granules are completely removed; a few eosinophilous cells remain with pink stained granules, but many show the large vacuoles only. In cases where the marrow had been affected by nucleic acid injections there are seen cells corresponding to the eosinophilous cells which contain granules stained ⁱⁿ ~~more~~ ^{distinctly} a purplish tint. In order to make certain that the granules seen after treatment with oxalic acid and other reagents were really the oxyphil ones, I carried out the method under the microscope as follows. A dry, heat fixed film was fixed on a slide with wax so as to leave a capillary space between the two. Weak eosin was run in and washed out with water, by means of blotting paper laid close to the edge of the cover glass. Having got a field with coarse and fine eosinophilous cells and cells with unstained granules (basophil), oxalic acid was then run in and after an interval, washed out. The eosinophil granules were found to disappear but the effect of the previous use of eosin lengthened the time necessary for their dissolution.

V Sodium Ethylate.

A 1% alcoholic solution of this was used to get the combined effect of alcohol and an alkali and I hoped

hoped that the action would be a more differential one than watery alkaline solutions. It was used at its boiling point and the films restored and stained as before described.

Results

The effect was practically the same as the alkaline solutions, the coarse oxyphil granules are little affected while the fine ones are mostly removed. The hemoglobin of the red cells is only partially preserved.

As to the interpretation of the results obtained by the use of dilute alkalies and acids one has to consider what bodies are likely to be present in the cells which would be soluble in these media and the nucleo proteid group is the one that naturally presents itself.

Against this view the only point of any apparent value that can be urged is the basicity of the granules as shown by their affinity for acid stains. This objection is less formidable than it appears, for it is not a question of absolute acidity and alkalinity that we are dealing with but a question of relative capability for superimposing acid or basic bodies to an already complex molecule. A good example of what I mean has already been cited in the case of the heads

heads of spermatozoa.

In the case of the eosinophile granule the nuclein is probably firmly united to a large amount of albumin and this, though weakly acid, reacts to acid dyes while still retaining its hold on its nuclein radicle. In support of the nucleo proteid nature of their composition is their behaviour to the reagents used as described above. Thus- the two forms are insoluble in alcohol and ether. Both forms are partially removed by acetic acid and Milroy has shown that the nucleo proteid of the pancreas is soluble in acetic acid at a ^{concentration and temperature} point when simple albumins and globulins are coagulated. The effect of alkalies shows that there is some distinctive difference between the two forms which may perhaps depend on the nature of the inorganic salts which are blended with the proteid part. The persistence of a few granules of one or other form after acids or alkalies points I think to differences in the rate of fixation and unequal distribution of the watery parts in marrow films. If the fine form of granule had been completely removed while all the coarse ones were unaffected, the idea might be deduced that they were stages in development of one form ; but all the

the work of this paper tends to show that they are separate and distinct varieties ^{but having however} ~~but have~~ the same kind of origin and ^{being} ~~are~~ allied in nature.

The amphophilous and neutrophilous granules are removed to a much greater extent by acids and alkalies than the coarse forms.

There are many points which might be mentioned here as having some influence in supporting the conclusion that these are bodies of nucleo albuminous or nucleo proteid nature and arise from the nucleus.

⁴³ Thus Unna and other dermatologists believe that the eleidin granules in the skin are nuclear in origin.

⁴⁴ Carlier has shown the great probability of the nuclear origin of the zymogen granules of the cells of the newt's stomach. *Both those types of granules have many points in common with the oxyphil ones in leucocytes esp. as regards reaction.*

Many mucins are known to be nucleo-proteids and in goblet cells there is an evident connection between the mucigen granules and the chromatin of the nucleus. In the eosinophilous cells in hen's blood the ripest cells, e.g., those which are seen on the eve of ^{breaking} ~~making~~ down, are very poor in chromatin.

Although the nuclei of many leucocytes remain rich in chromatin while others with granules at apparently the same stage of development are relatively poor, this

this does not argue against the nuclear origin of the granules, for the chromatin (nuclein) is most probably only the storehouse from which the material is taken to produce the granules and not the actual molecular mechanism by which these granules are produced, and hence the output may be uniform as far as we can see whether the nucleus is rich or poor in nuclein. ✓

But when we can experimentally produce an exhaustion of the granular cells so that their nuclei become very poor in chromatin and their granules diminished in quantity and to some extent altered in reaction we have surely some grounds for believing that there is here an intimate interchange between the nucleus of the cell and its cytoplasmic granules. It is very probable that both the fine and the coarse oxyphil granules belong to the same class, the main difference probably being in the organic salts present, ~~and~~ ~~and~~ the firmness of the union between the nuclein ^{and the relative amounts of nuclein + albumin.} part and the albuminous part. This is perhaps the most appropriate place to advance an hypothesis and explain in this way the possible nature of the amphophile reaction of the fine oxyphil granules of rabbit's blood. The union here is probably a loose one and the nuclein part or the albuminous part is able

able to link on the basic or acid coloured body without severing its connection with its partner, whereas in the coarse oxyphil granule the union is such a firm one that the whole molecule reacts as one composite group of atoms which is able to link on only the acid dyes because its own reaction is so weakly acid as to be basic to most acid stains.

The neutrophilous granule is midway between these two in the firmness of the union of its constituent parts.

I shall now give the chief points connected with the experiments on animals.

These experiments were carried out in order that one might find out the relationship between the granules and the nucleus after the action of a constituent of the latter viz - nucleic acid.

It was imperative to find the connecting link between the general action on the phosphorus ~~excretion~~ metabolism as a whole and the specific intracellular metabolic changes which were the primary factors in producing the general effect.

That is to say, is there a specific intracellular change at the great formative centre for the oxyphil cells after the action of nucleic acid?

EXPERIMENTS ON ANIMALS.

These experiments have reference chiefly to the effect of subcutaneous injection of nucleic acid on the granular leucocytes as regards,

- (a) their distribution in blood, marrow, and lung capillaries.
- (b) the number of granules per cell
- (c) the staining reactions of the granules
- (d) the staining power of the nucleus.

The animals used were young healthy adults and the injections did not seem to produce much systemic disturbance, although death followed in the case of a small guinea-pig after injection of .2 grm. nucleic acid.

The nucleic acid which was used, was got from Kossel's laboratory by the kindness of Dr T.H.Milroy. It had been prepared from thymus glands and hence was of that variety of nucleic acid which is derivable from leucocytes themselves. It was considered very important to have the injection neutral, for in herbivorous animals such as rabbits the serum does not seem to have the power of maintaining an equable degree of alkalinity and the condition of the serum must have an important influence on the leucocytes.

It was injected as nucleate of soda - made by

by warming some distilled water to temperature of the hand and then adding the acid and neutralizing the fluid with sodium carbonate: the fluid was then *allowed to* ~~let~~ settle, measured and a fixed amount injected under the skin of the abdomen. Care was taken that the fluid was not heated above 40°C when neutral or slightly alkaline, as under these conditions the nucleic acid is split up. For this reason, as well as ~~for~~ *from* the fact that nucleic acid is itself an antiseptic it was not deemed necessary to sterilize the fluid before injection and there was never at any time any sign of septic infection at the points of inoculation.

In counting the number of leucocytes in rabbits, the blood was taken from the central vessel on the posterior aspect of the ear - the part being previously shaved and cleaned. In guinea-pigs and dogs it was taken from the pad of the foot. In hens - from the upper aspect of the toes.

Blood films were taken on No. I cover-glasses cleaned by sulphuric acid and potassium bichromate - finishing off with water, alcohol, and drying over the flame.

In taking the films Ehrlich's special forceps were used to draw the covers apart after allowing the

the drop to spread well out.

Marrow films or "daubs" were taken as described by Professor Muir, ⁴⁵ - that is allowing the cover glass to touch the marrow and then withdrawing it without making streaks of the marrow.

The marrow was taken from the femur as low down in each case as possible.

A. Experiments on Rabbits.

The first series of experiments which will be described was done on a litter of young rabbits - all of the same age and size.

Rabbit I. was used as a control.

The white corpuscles were found to average 5,400 per cmm, the reds 4,560,000. Blood films were taken and the animal killed by a blow on the back of the neck.

Specimens of marrow and lungs were fixed in Hg Cl_2 and blocked in paraffin. Films of the marrow were also taken and fixed along with the blood films in:-

- (1) Formalin Vapour
- (2) Heat. (1) 120°C for two hours
 (2) same for ten minutes.
- (3) Hg Cl_2 in normal saline (Muir's method)
- (4) Alcohol and ether equal parts for $\frac{1}{2}$ an hour.

hour.

Of these methods formalin vapour was found to give the best results for the purpose of the work. The film was placed on a piece of cardboard perforated ^{by} a hole to suit, and then put over a small flat glass dish containing ordinary formalin. The time given was the same in each case - three minutes.

Results of examination of Rabbit I. (control) Marrow films.

The following descriptions are the result of the examination of films fixed by the above mentioned methods and stained in various ways. Chenzinsky's solution was found to give very good results. It consists of an intimately blended mixture of eosin and methylene blue. The fluid was filtered afresh each time before use and the staining was done in the incubator at 40.C for six to eighteen hours.

Ehrlich's triple stain, Stöhr's eosin and haematoxylin, and other stains were also used. The colours mentioned here refer to eosin and methylene Blue staining.

(1) The amphophilous cells of the marrow are seen to be much more numerous than any other form. Their contour is as a rule well defined although occasionally one sees a cell with indefinite margin and

and scattered granules. This, however is rare compared to cases where there is no such breaking down. The nuclei show all changes ^{in ~~with~~} ~~in~~ shape from a perfect oval to an indented and convoluted form and are stained fairly deeply ^{with} ~~in~~ basic dyes. The cell groundwork in which the granules are placed is stained ^{slightly} pinkish in eosin mixtures and one can see through the nuclear matter pink spots probably the cytoplasm seen through vacuoles in the nucleus. One never sees as in the ⁿuclei of erythroblasts, distinct red granules.

The amphophilous granules are highly refractile bodies which fill the whole cell.

Occasionally a cell is seen with comparatively few granules but this is rare compared to the frequency of cells which seem quite full of them. These granules in the rabbit seem fairly easily discharged so that vacuoles are left in the cell.

II. Eosinophilous cells of marrow.

These vary in size from a form about as small as a large erythroblast up to forms larger than the amphophilous cells.

The shape of the nucleus is never so easily made out as in the finely granular ones although it

it stains as a rule fairly deeply. It also shows indentation and may be convoluted but not to the same extent as in the finely granular.

The granules are very distinctive - they are round and regular in contour, of a dull red colour and are nearly always very closely packed - so that the cell presents a compact rounded appearance as a whole - and the granules are very seldom found scattered about. Occasionally one sees a granule stained with the basic stain in a cell where the great majority are red.

These cells are fairly numerous but it is difficult in marrow "daubs" to know the exact proportion in relation to the finely granular, but one may say at least 7 of the former to 100 of the latter. The granules with the purest red tint seem to be present in cells where the nucleus is almost invisible.

III. Basophilous granular cells of the two types are present but in lesser amount - especially ~~as to~~ the coarse variety.

IV. Erythroblasts show well stained nuclei. They are not quite homogeneous but have faint acidophil granules

granules - these are seen centrally or eccentrically placed.

The cytoplasm of the erythroblasts is stained of a more or less pale rosy red - it is apparently homogeneous and very similar to that of the erythrocytes ~~blasts~~.

The erythroblasts vary in size - some being smaller than the erythrocytes, some of the same size and other larger. The nuclear matter of the megalo-blasts is more faintly stained than the normoblasts.

V. The erythrocytes are seen with the central dell and are stained a pale rusty red with faint tinge of blue.

VI. Other marrow cells.

Some large cells with relatively large nuclei stained faintly blue. The faint rim of cytoplasm is stained deep blue and the nucleus is not quite homogeneous, others very similar but smaller have very little cytoplasm at all.

In films stained with methylene blue alone the coarse oxyphil cells are seen with unstained areas corresponding to the granules; while the

the amphophilous granules are stained violet. The nuclei of the latter is more deeply stained than the former. Transition changes can easily be made out from the myelocytes with round or oval nucleus to those with indented nuclei and fine violet granules and during this transition the nucleus seems to increase in basophilic tendency.

If, after staining with methylene blue and examining as above, one stains in eosin for half a minute and again examines - the granules which previously were stained with the blue still retain it while the "vacuolated" cells stand out as coarse oxyphilous, *their granules being stained of in the pure eosin tint.*

Orange (G) in saturated watery solution stains the amphophil granules a deep orange tint while the coarse oxyphil ones are faint yellow. *This makes a very good differential stain.*

Fuchsin - here the difference is not so marked as with orange, but the fine oxyphil are ^{of a} more violet while the coarse oxyphil are ^{of a} more purple, *tint.*

Blood of Rabbit I.

Here the amphophilous cells are seen as

as compact spherical cells, crowded with granules which in triacid are stained a reddish purple. The nucleus occupies a great part of the cell and is stained a fairly pale green.

In some cells the granules seem to possess slight differences in tint.

The coarse oxyphilous cells are much fewer in number. The main difference between them and the former being in size and tint of the granules which are much larger and stain purple with triacid - the nucleus is often deeply stained. The granules are crowded very closely together. The lymphocytes present homogeneous nuclei which ^{are} ~~is~~ more basophil than ~~that~~ ^{these} of the granular cells though less so than that of their own cytoplasm. These points are brought out best in fixation by heat and formalin vapour and in staining by Chenzinsky's solution, Triacid, and Stöhr's weak eosin with Haematoxylin.

Marrow Sections fixed in corrosive sublimate, cut in paraffin and stained with Chenzinsky.

Low Power. A considerable number of fat-cells are seen and giant cells. Bright pink spots are thickly scattered over the field corresponding to the coarse eosinophil cells.

cells.

X (480) The granules of the coarse eosinophil cells are distinctly seen, they frequently occur near fat cells and their nuclei are too faint to be made out with this power.

Large numbers of erythroblast nuclei are seen and evidence of mitosis occurs now and again. The amphophilous granules cannot be made out nor the basophilous but nuclei can be seen stained faint blue which must correspond to these two classes of cells.

Giant cells with well stained nuclei seen here also. The blood vessels are not prominent.

X (1100). ($\frac{1}{12}$ oil immer. oc 8)

In any single field there are to be seen very numerous amphophilous cells containing numerous fine blue or violet granules. Their nuclei are not very deeply stained in this case (Chenzinsky's solution). In the same space there are always a few coarsely granular acidophil cells to be seen - the number varying from two to six or seven. In these the granules are crowded closely together and the nucleus is usually seen to one side of the mass of granules -

granules - there is no distinct cell ground work to be made out. The granules stain a uniform dull red colour and the nucleus frequently presents a distinct green ~~cell~~ membrane with a central green nucleolus and a clear space between these two.

Erythroblasts and other cells present well stained nuclei, mitotic figures being fairly common in the former.

In marrow sections stained in eosin and Löffler's methylene blue, practically the same appearances occur. The amphophil granules are not stained by this method and it is difficult to classify all the nuclei that are seen. The eosinophil cells are well stained - the granules being bright pink and the cell nucleus faint blue with darker nucleoli.

Rabbit II.

Here only one injection of nucleic acid (.5 gm in neutral solution) was given.

The leucocytes were not examined specially as to their numbers but a count made shortly before killing, at 18 hours after the injection showed 4,000 and the number present in the blood films then taken

taken corresponds to this somewhat low figure.

Marrow films were taken as before described.

Results of examination.

Marrow films fixed with formalin vapour or ~~heat~~ and stained with Chenzinsky's methylene blue and eosin show: -

I. Amphophilous cells occur in all parts of the films. They are larger and looser in structure than normal, and there is a tendency for the granules to become loose and scattered. Many of the cells possess very few granules and in those that are seen the tints of the granules vary remarkably - many of them being violet and others pink.

In a great many cells unstained areas are seen corresponding to the granules. These spots seem to be surrounded by a ring of blue stained substance as if the original granule were composed of a central and peripheral part (as Ehrlich believes is the case in some) and that only the peripheral part had become stained.

These apparent vacuoles in the ~~the~~ cells must be due to a change in reaction for very few such appear in the control specimen and they are seldom

seldom seen in specimens stained with triacid.

II. The coarse eosinophil cells are diminished in number, but not so markedly at this stage as ~~seen~~ in later cases. The chromatin of their nuclei as well as that of the foregoing shows a distinct diminution in staining power which is partly due to increased size of these nuclei. The eosinophil cells are much looser in structure and the granules take on different tints in single cells - the tendency being to a more basophil reaction.

III. Erythroblasts and other cells are stained as in the control films.

Blood films Rabbit II.

(1) Amphophil. There seems to be a diminution in the number of leucocytes. The majority of those seen are larger than in the control - most of them averaging one-fifth more in diameter. They are hazy in outline and have pale, swollen nuclei and a diminished number of granules per cell. The granules seen are sometimes more basophil, sometimes more acidophil than normal. In some cases the granules seem to have gone entirely leaving a homogeneous faint pink groundwork in which unstained areas,

areas, corresponding in size to the fine granules, occasionally occur. Besides these there are amphotophilous cells present which are apparently unaltered.

The eosinophilous cells are so few that it is difficult to get specimens showing them. In a film stained with triacid mixture they appear as cells somewhat larger than normal with pale chromatin and numerous granules which stain uniformly in this solution. They seem to be but slightly altered in reaction in the blood at this stage. The changes in the blood of this rabbit are of the same nature as in the next, v. (Rabbit III.).

Marrow Sections.

The appearances seen in these correspond to that described in the films. Specimens of this rabbit's marrow accompany the paper.

Rabbit III.

The following table embodies the chief points connected with the treatment of this rabbit. (III.)

Rabbit III.

<u>Date</u>	<u>Hour</u>	<u>No. of leucocytes per cmm. of Blood.</u>	<u>Remarks.</u>
Mar. 29	9.30 am.	5,500	Animal fed sometime previously.
"	10.45 am	-	Injected .4 grm nucleic acid (in 5 cc soln.)
"	5.30 pm	6,100	Probably the hypoleucocytosis had passed.
Mar. 30	10 am	6,600	Probably represents a hyperleucocytosis.
"	1 pm	-	Injected .5 grm in 15 cc
"	6 pm	3,400	Hypoleucocytosis - note time - (5 hours since injection).
Mar. 31	10 am	5,600	

Mar. 31	5 pm	7,600	
Apl. 1	11 am	6,800	
-	3.45 pm	-	Animal killed.

In short, the animal showed a distinct though moderate reaction to the nucleic acid injections, ^{It} and was

killed at 50 hours after its last injection. ^{The} ~~the~~ first injection was given 26 hours before the first one. Marrow and

blood films were taken and fixed as before.

Results of examination.

Marrow Films.

In a specimen fixed with heat or formalin and stained with Chenzinsky's solution:-

I. The first thing that strikes one again is the very loose distribution of the amphophilous granules - there are few or none so compact and well defined as in ^{Rabbit} I. They have also changed in reaction so that now they take on more of the blue tint of basophilous cells - a few granules are left, however, which show the violet reaction. Many of the amphophilous ~~cells~~ show unstained areas which must correspond to vacuoles - this appearance is comparatively rare in normal marrow films. The nuclei of the amphophilous cells ^{are} is very pale.

II. AS to the coarsely granular eosinophil

eosinophil cells they have almost completely disappeared and in those left the granules show a tendency to take on the basic stain and the nucleus is very poor in chromatin. These cells also frequently show vacuoles.

III. The basophilous cells are still present but are now almost indistinguishable from the amphophilous. The erythroblasts seem less numerous compared to the amphophilous and the cytoplasm is in many cases stained with methylene blue. In the nuclei the same appearance of red granules is seen. Mitotic figures are fairly numerous.

IV. Other marrow cells - small and large cells occur with large faint blue nuclei and rim of deeper blue cytoplasm around containing no granules. These are not so frequent in proportion to the granular form as in the control films.

There are also cells exactly similar to the amphophilous which contain no granules but some small vacuoles.

Blood films of Rabbit III.

These were taken from the ear shortly before killing. Here the ordinary polymorphic leucocytes

leucocytes present a marked contrast to those of the control. The nucleus is paler and seems swollen, and its outline is less distinct. It is also less convoluted. The granules are very much fewer per cell than normal and the whole structure of the cell is loose and swollen looking. The cell protoplasm extends beyond the distribution of the granules and is seen as a fine homogeneous basis. Some cells are seen containing as few as 5 or 10 granules, grouped within the angle of the nucleus, while some cells contain almost as many as usual and others which undoubtedly correspond to the finely granular cell contain none at all.

The partial disappearance of the granules is more marked in films fixed with formalin vapour but is quite distinct also in those fixed with heat and other ways and stained ^{with} in various dyes. The eosinophilous cells are very few in number, and in those seen as in the case of the finely granular cells there is a tendency to take on the basic stain in some granules. The lymphocytes and red cells are well seen and show no change.

Marrow Sections.

Marrow Sections.

(x 80) A large amount of parenchyma is seen with collections of red blood corpuscles showing distinct congestion. The spots indicating the presence of eosinophil cells are not nearly so numerous as in I. The nuclei do not seem so well picked out and there is a tendency towards diffuse staining with eosin.

(x 480) The cell nuclei and protoplasm take on nearly the same tint. There are very few eosinophilous cells to be seen - the diminution being as marked here as it is in the case of the films.

Accumulations of erythrocytes in the parenchyma point to distinct congestion.

The nuclei of the erythroblasts can be easily made out as they stain much more deeply than the nuclei of the myelocytes. Giant cells are fairly numerous and large.

(x 1100) There are very few granular cells of any kind to be seen, - the whole preparation shows a diffuse undifferentiated eosin stain. The eosinophil cells which are well stained are very scanty - their nuclei are almost invisible and the granules seem

seem swollen, more loosely arranged and darker than normal.

The nuclei of the erythroblasts are not so well stained as in the control and the nuclei of what must have been amphophilous cells are swollen and pale.

Practically the same appearances are seen in specimens stained with eosin and Löffler's methylene blue - Here the nucleoli are seen to be stained with eosin as well as the other parts.

These changes point to a profound alteration in the marrow - which is of the same nature as "cloudy swelling," but whatever name be applied to it, it is evidently an exhaustion of the tissue as shown by the loss of chromatic staining and of cell granules.

The diminished number of eosinophils may be due to an altered distribution but there is distinct evidence of their altered character as seen in the loss of their chromatin and tendency to basophil staining on the part of the granules.

Kanthack and Hardy showed that eosinophil cells accumulate in the connective tissue spaces

places
spaces around where bacteria have been injected. I examined films of the areolar tissue from the places where the nucleic acid injections had been made but could find no evidence of such accumulation in these cases.

Rabbit IV.

The point aimed at in this rabbit was to procure marrow at a stage where the exhaustion produced by the nucleic acid would be passing off.

The following table shows the treatment.

Rabbit IV.

<u>Date</u>	<u>Hour</u>	<u>No. of leucocytes per cmm. of Blood.</u>	<u>Remarks</u>
Mar. 29	10 am.	6,100	
"	12 n'n		Injected .2 grm nucleic acid (in 2½ cc)
"	5.45 pm	4,500	Moderate hypoleucocytosis
Mar. 30	11 am	5,100	
"	12 noon	6,100	.5 grm. nucleic acid (18 cc)
"	5.30	3,700	Hypoleucocytosis
Mar. 31	11.15 am	6,600	Relative hyperleucocytosis.
	12.45 pm	-	Injected .5 grm. in 9 cc.
	5 pm	3,200	Again a hypoleucocytosis
Apl. 1	10 am	5,400	
"	12 noon	-	Injected .5 grm in 10 cc
Apl. 2	1.30 pm	3,600	This may have been a secondary hypo-l.
- 3	10 am	6,000	
- 4	10 am	5,600	
	2.30 pm		Animal killed

In this rabbit the time elapsing between the last

last injection and the killing of the animal was 75 hours as compared with 50 for III. The amount injected was nearly double that of the other case, but spread over several days.

Results of Examination.

Marrow films. (Chenzinsky)

I. The amphophilous cells are very numerous. Their nuclei are still very faintly stained but slightly more deeply than in III. Their contour is much the same as in I. many of them being indented and homogeneous in appearance. The granules differ from those of III in being distinctly more acidophil. They have the same loose arrangement so that bearing in mind the irregular, ill defined outline, the cells appear as if they were either breaking down or beginning to secrete after having broken down.

Some of the granules are pink, some violet and some blue but the majority are *distinctly acidophil* pinkish.

II. The coarse eosinophilous are more numerous than in III but much less so than normal. They have much the same appearance as in III but here also there is a greater tendency to the normal acidophile reaction though there are to be found numerous

numerous examples of the cells containing blue, violet, and dull red granules, all in the same cell. Loose granules of the same kind also occur. The nuclear matter in many of these eosinophilous cells has practically gone.

III. Basophilous cells seem less numerous than in III.

IV. Erythroblasts are more numerous and better stained (both nucleus and protoplasm) than in III. All the different sizes are seen, some undergoing mitosis.

V. Large and small cells are also seen resembling lymphocytes, with large faintly stained nucleus and a narrow zone of more deeply stained cytoplasm around.

These are decidedly more numerous than in III. Other cells are seen containing more protoplasm and oval or even convoluted nuclei in the cytoplasm of which are a few granules.

Blood films of IV. show that the granules (amphophilous) are more numerous than in III but still less than normal. The nucleus is very pale and there is a remarkable amount of variation in the

the tints of granules in single cells. The cell seems to have regained its normal size. Lymphocytes and red corpuscles show normal staining.

Marrow Sections.

x 80 - congestion is still apparent or even greater than in III. The marrow has lost its tendency to diffuse staining and has approached nearer to the appearance seen normally.

There is a very distinct increase in the parenchyma, due to an increase in the cellular elements.

The eosinophil cells are still greatly diminished in number.

x 480. In many respects the marrow is seen to be more normal as regards the character of its cellular elements. The eosinophil cells are still few, but the granules are more discrete and the cell outline is better defined than in III.

The nuclei of the amphophilous cells show a return of their staining power. ^{their} ~~its~~ contour is better defined. Erythroblasts are seen in clusters with distinct homogeneous blue nuclei.

Capillaries can be seen with numerous erythrocytes in them and a few erythroblasts and leucocytes.

leucocytes.

Fat-cells are small in size and not so numerous as in the control rabbit.

(x 1100) The specimen still shows some of that diffuseness of staining seen in III. but the nuclei as a whole are much better stained. The nuclei which have most recovered are those of the erythroblasts which have returned to their normal condition, although their outline is still a little indistinct. Frequent mitotic figures occur in them.

Eosinophilous cells are seen in greater numbers than in III but their nuclei are still pale and not nearly so well defined as in I. Amphophil cells are now seen with fine granules which stain violet or pink.

In the Löffler methylene Blue and eosin specimens the nuclei of the erythroblasts are well stained while as above the nuclei of the granular cells are faint. Fair numbers of eosinophil cells are seen and in some the nuclei seem as well stained as normal. The amphophil granules are not stained by this method.

method.

With reference to the changes in the distribution of leucocytes in the lungs it need only be mentioned that there was found to be a distinct increase of blood corpuscles with a relative increase of the whites. These changes are similar to those found by Goldscheider and Jakob and (independently) by Professor Muir.

I could not make out the relative increase of the different varieties of granular cells in the lung capillaries.

EXPERIMENTS ON DOGS.

There are two interesting points in connection with dogs that would lead one to use them in this connection. Stadthagen⁴⁶ and Gumlich⁴⁷ have shown that in them, the administration of nuclein leads to a greatly increased P_2O_5 excretion. The other point of interest is the statement of Sakaroff⁴⁸ that in dogs, the eosinophil granules arise from erythroblasts by a phagocytic action on the part of the leucocyte.

In spite of these inducements, however, it turned out that dogs were not nearly so suitable as

as rabbits for showing changes in their cytoplasmic granules. The fine oxyphil granules of the dog's leucocytes are so closely packed that the cell is almost homogeneous unless when stained with the tri-acid mixture.

Again, the coarse oxyphil cells are far from numerous in either the blood or marrow.

As, however, the results got were interesting and to some extent confirmative of the changes seen in rabbits a short account of them may be given.

The animals used were both of the same age (two months), and each weighed about two kilos.

The treatment was practically the same as described for the rabbits.

Dog A.

Date	Hour	Nos. of leucocytes.	Remarks.
Mar 1st	-	13,800	-
" 2	10 am	11,000	-
" "	11 am	-	Injected subcutaneously .35 grm. nucleic acid.
" "	3.30 pm	17,450	-
Mar. 3	9.30 am	21,000	Hyperleucocytosis.
" "	10.20 am	-	Injected .15 grm nucleic acid.
" "	4 pm	17,400	-
" "	5 pm	-	Injected .3 grm. nucleic acid.
Mar. 4	3 pm	-	Animal killed.

It would have been better if a longer period had been

been allowed to elapse before the animal was killed so as to get the "exhaustion" stage of the granular cells ^{seen} ~~got~~ in the rabbit at fifty hours. Here the *animal was killed after twenty two hours.* ~~period was twenty-two.~~

Dog B. was killed ^{in order that} ~~for the sake of~~ its marrow ^{might be used} ~~to get~~ as a control. Specimens of the spleen were also taken from each. *but the changes seen do not bear on this paper.*

Results of Examination.

Blood films of (A) taken before injection of the nucleic acid, fixed with heat, and stained in the triacid mixture show the following:-

I. Finely granular oxyphil cells are seen, having a cell body crowded almost to opacity by very small darkly stained granules.

The nucleus is a convoluted or simply vent elongated mass. It is apparently composed of a fairly dark green core edged all round by a thin clear green zone.

well

The whole cell is small, ^{well} defined, and spherical.

II. The coarsely granular oxyphil cells are very few in the blood of this dog - thus I could only find ten in one large film which contained over

over sixteen thousand leucocytes of all kinds. The granules are discrete, rounded bodies, of moderate size and take on the purple stain with the triacid mixture. The nucleus is of pale green colour, rounded or indented and occupies about one half of the area of the whole cell.

III. Lymphocytes present no special peculiarity.

IV. Large lymphocytes with indented nuclei occur fairly often.

Many of the red cells show the appearance of giving rise to blood platelets as described by Arnold.

When the dog's blood is stained with Chenzinsky's solution the finely granular cells present a perfectly clear, homogeneous, unstained cell body while the coarse granules take up the eosin tint. The nucleus is seen to be lobulated and stains a bluish green colour.

In films of the same dog taken after injection of nucleic acid - a few hours before death, the following differences are observed:-

i. The finely granular cells have in a great many cases enlargement of the nucleus, as shown

shown by an increase of the thin green zone before described — the outline of the nucleus is hazy and difficult to differentiate from the cytoplasm. These cells contain the fine granules - one cannot say whether they are altered in character or not. Besides these, there are normal cells corresponding to those found before injection, but also a considerable number of cells which seem to be neutrophilous but from which the granules have disappeared. They present a rounded hazy outline - the nucleus and cytoplasm are merged into one another so that the outline of the former is lost, but the central core is still left and it is this that allows the cell to be classified. There are no traces of granules in the cell, the cell body being stained a homogeneous grey or pinkish colour.

ii. The eosinophilous cells in the blood are still comparatively few in number but are apparently increased - they form about .5 per cent of the total number of leucocytes.

In the cells which were found, the following changes were seen - The whole cell structure seems looser - the granules are set more apart and vacuoles are seen in some, groups of free granules occur;

occur: without any evidence of a nucleus and in those cases the granules vary in tint; some of the cells show very pale nuclei.

iii. The lymphocytes and other cells show no apparent change.

Marrow films of dogs A. and B.

1. In the control specimen^B, the following varieties of cells are to be seen:-

I. Eosinophilous cells with moderately large granules and kidney-shaped or convoluted nuclei which occupy the greater part of the cell area. When stained in triacid, the granules appear of a reddish purple - but similar cells are seen with granules stained with orange and these seem very clearly related to these cells with the purple granules. The granules are very numerous in each cell as a rule and are seldom seen free. They show slight variations in size in some cases.

II. Finely granular oxyphil cells are not very numerous. They are usually large cells with large oval or indented nuclei. The granules are finer as a whole than the foregoing and stain a deeper purple. They are not crowded together as a rule

rule but are found scattered through the cell ground-work which is stained a dull violet colour.

These cells are very few compared to the large numbers of fine oxyphil cells found in the marrow of rabbits or guinea pigs. Both forms of oxyphil granules stain in glycerine solutions of eosin.

III. Frequently one sees cells corresponding in size and shape of nucleus to the fine oxyphil cells in the blood but there are no granules to be made out even in triacid staining. In this case the cytoplasm stains a dull homogeneous violet.

IV. Large cells are also seen with large rounded granules which are by no means very numerous thus the number in each cell can easily be counted and is usually four or six sometimes even twelve or more. They have rounded or oval pale green or violet nuclei (in triacid stained films) and the granules are dark violet homogeneous masses set in a cytoplasm which is also homogeneous and stains a dull violet colour. The nuclei are often indistinct as if degenerated. The granules vary in size, some being as large as the nucleus of an ordinary erythroblast and it is possible they are the results of

of degeneration of erythrocytes or erythroblasts which have been included in a leucocyte by phagocytosis. In one cell I found what was undoubtedly a freshly included erythroblast.

It is on appearances like those that Sakaroff states the theory of origin of eosinophil granules from erythroblasts by phagocytosis on the part of certain leucocytes but the cells are so totally different in size, structure of nuclei, sizes and numbers of granules from the true eosinophil cells that it seems impossible to believe that they develop in this way.

V. Erythroblasts are present in great variety many of them showing extrusion of nuclei and many megaloblasts are also seen.

2. In the marrow of Dog A. (after injection of nucleic acid) there are not many changes that can be described with reference to the appearances of the granules.

The eosinophil cells are seen in about the same proportion as in the control. This I think is due to the animal being killed too soon for the most marked changes occur in rabbits at fifty hours, whereas only twenty-two hours had elapsed in this case.

case.

however

There is ^{as above} distinct evidence of increase in size of the nuclei of the cells described under III. above. This form of cell, it will be noticed, corresponds to the fine oxyphil cell of the blood in everything except the distinct development of the granules. This increase in size is not in any way due to the method of fixation which was as far as possible identical in both cases - the films being put into the oven at 117° c in one case and 118° C in the other. It is unfortunate that formalin fixation was not used here as in the later work on rabbits - as it gives much sharper definition.

The coarse eosinophilous granules show a distinct tendency to take on more of the orange tint when stained in triacid than is the case in the normal marrow film.

The conclusions that can be drawn from the above descriptions are given at the close of the paper.

C. EXPERIMENTS ON GUINEA PIGS.

These were carried out on about half a dozen guinea pigs - also with a view to find out alterations in the characters of the granules but more especially to find out whether there was any difference in the

the solubilities of the oxyphil granules after injection of nucleic acid. As to the latter point it may be said here that although a long series of experiments were done on guinea pig blood films taken at the hypo and hyper-leucocytosis stages and also on guinea pig marrow after nucleic acid injection - there was no appreciable difference in solubilities obtained.

As to the effect of nucleic acid on the leucocytes of guinea pigs, there is not found the same instability of reaction which was got in the case of rabbits but the two series are not strictly comparable for I had not begun to use formalin fixation or the methylene-blue-eosin stain and the chief method used was heat fixation and staining with watery eosin and haematoxylin, a method which raises the acidophil tendency of the oxyphil granules so much that minute differences are inappreciable.

The method of fixation by heat was employed in order to test the solubility of the granules after the action of different reagents as it is the only one that can be used for this purpose.

The normal appearances of the granular cells of the blood of guinea pigs may be shortly stated as follows:-

I. The coarsely granular oxyphil cells are numerous in both blood and marrow; they are usually crowded with their granules which are very uniform in

in size, shape, and staining reaction in each cell. The nucleus is rounded or kidney-shaped in the marrow - more convoluted in the blood. The cells are much smaller, more compact, and better defined in the latter situation than ⁱⁿ the former.

II. The finely granular oxyphil cells are very numerous in blood and marrow. The granules are very discrete and strongly acidophil when compared to the corresponding cells of the rabbit. The nuclei resemble those of the above and, as in the foregoing, they also stain fairly well especially with haematoxylin. In the blood these nuclei are very polymorphic and the chromatin forms massive knobs on the sausage-shaped nucleus - occasionally this is so marked that there appear to be four or five nuclei in one cell, but some of the cells have nuclei which are oval or kidney-shaped and here the granules are just as well developed as in the others. The nuclei occupy from one-third to one half of the cell area.

III. Basophil cells with fine and coarse granules are very numerous in the marrow.

IV. Other granular cells described by Kurloff have granules staining with nigrosin and there are still others frequently seen in guinea pig marrow

marrow which stain very faintly with orange and are easily mistaken for cells with vacuoles. Besides these granular cells, lymphocytes and large uninucleated cells are seen with apparently homogeneous protoplasm. Some cells are seen which have polymorphic nuclei, not so convoluted as in the majority of the fine oxyphil cells; The cytoplasm here has a basophil tendency and the nucleus occupies more of the cell area than in amphophil cells.

It is difficult to believe as some do that these are transition changes from the large lymphocyte to the neutrophilous granular cell for the following reasons:-

(1) The neutrophilous cells have nuclei varying in shape from the oval indented to the orthodox convoluted form ^{although} but the large lymphocytes have some nuclei which are also considerably convoluted, ^{there is} ~~without~~ ^{no} ~~any~~ trace of granule formation.

(2) The nuclei occupy relatively much more of the cell in large lymphocytes than in the neutrophilous.

(3) The cytoplasm of the lymphocyte is more basophil than that of its nucleus while the converse holds in granular cells.

cells.

After nucleic acid injection there were some alterations in the relative numbers of the granular cells to the non-granular in the blood. Thus in the stage of hypoleucocytosis the proportion of granular to non-granular forms was as 60 to 40 while in the films of the same guinea pig taken before injection of nucleic acid the proportion was 70 : 30. In the stage of hyper leucocytosis the relative percentages were 76% amphophil, 22.5% non-granular forms and 1.5% eosinophils. These proportions were got by counting the numbers of the different varieties in stained films and might of course as well mean a relative increase or decrease of the non-granular forms as a decrease or increase of the granular ones but the latter is the more likely to occur.

Marrow films - (control guinea pigs).

These were fixed with heat and stained in eosin and methylene blue.

I. Eosinophilous cells with coarse granules are well seen. They are large, well defined cells with elongated or kidney-shaped nuclei and are crowded with dull red granules which are uniform in

in size and depth of stain in each cell. The cell groundwork is unstained.

In a few cases these cells have been broken down and the granules scattered all round.

II. Neutrophilous or fine oxyphil cells are seen containing very numerous pink granules which are very discrete and well defined. The nucleus is often of a convoluted sausage shape and occupies a considerable part of the cell. The cell ground work takes on a bluish or pink tint.

Many of these cells have been broken down.

III. Other marrow cells are the basophilous forms, fine and coarse, the granules of the latter varying much in their shape, ^{normo}erythroblasts, megalo-blasts and the large lymphocyte-like forms.

Marrow films of "G" Guinea Pig.

This guinea pig had .3 gm nucleic acid injected subcutaneously

In this case nucleate of soda had been injected (.3 grm) and the animal killed about twenty hours after the last injection.

When stained with methylene blue and eosin, the first that strikes one is the large amount of blood that must have been present, pointing to distinct congestion, and the same is borne out in the marrow sections.

sections.

I. The eosinophilous cells show alterations in tint in single cells, some being darker than others but none show that marked change seen in rabbits. The cell as a whole seems looser and is apparently easily broken down.

II. The neutrophilous cells are very poor in chromatic staining and the granules show indications of the same changes in reaction but not markedly.

From the presence of so many red corpuscles it is difficult to estimate the relative proportions of cells present, but the number of eosinophils does not seem to be diminished.

In another guinea pig (C) the marrow films show increase in size of the nuclei and an apparent increase in the number of granules in each cell. The coarse oxyphil cells are very numerous but probably not much more than normal. With the stains used at that time it is impossible to distinguish variations in tint of the granules.

*Here also. From
nucleic acid was
injected.*

D. EXPERIMENTS ON HENS.

These experiments were undertaken primarily for the

the purpose of examining the excretion of phosphorus, alloxur bodies, etc., after injecting nucleic acid.

It was found, however, very difficult to collect all the excreta and, besides there were very wide daily variations so that it was impossible to get a state of nitrogen-equilibrium.

One point of interest may be noted, viz., the large amount of phosphorus excreted compared to the nitrogen - thus in one estimation the amount of nitrogen (estimated by Kjeldahl) was 1.24 gm. while the P_2O_5 was .93 gm. and other estimations give similar results. This is a significant fact when one remembers that the blood of the bird is rich in nucleated elements and that the eosinophil cells constitute at least 90% of all the leucocytes.

the following table shows the treatment of the hen experimented on - the other being merely used as a control.

"Nucleic Acid" Hen.

Date	Hour	Nos. of leucocytes	Nos. of red corpus.	Injections of Nucleic Acid.
July 12	noon	63,125	1,932,000	
" 14	"	56,250	1,297,000	
" 18	"	65,625	1,862,000	
" 19	10.30 am	48,437	1,311,000	
" "	2 pm	-	-	.1 gm
" "	5 pm	80,000	2,362,000	
" "	7 pm	-	-	.9 gm.

July 20	noon	130,000	2,920,000	1 grm
"	3 pm	90,000	2,000,000	
"	3.30pm	-	-	animal killed.

It will be noted that there was an effect on both forms of blood elements.

Blood films were taken before and after the injections. They were fixed by heat and stained in Eosin-indulin-aurantia in glycerine solution (Huber)

The normal blood shows coarse eosinophil cells where the granules are distinctly spindle-shaped, with occasionally the appearance of a vacuole in the centre. Some of the cells are compact, and seem quite opaque from the number of these bodies which lie in the cell side by side, so that at the circumference they resemble the staves of a barrel.

In some, however, the cell is loose in structure as if broken down and in these the nuclei are very pale, indeed almost gone - its shape being only indicated by the distribution of the granules.

The nuclei in these coarse eosinophil cells are oval or kidney-shaped, never convoluted in the same way as those of mammals.

II. Fine oxyphil cells occur in slightly less proportion than the above the average number being four of the ~~former~~ ^{*fine granular*} to six of the ^{*coarsely granular ones*} latter. These

These finely granular cells have nuclei which are often more indented so that when placed with the indentation upwards, it looks as if there were two small round nuclei at opposite sides of the cell - these nuclei are fairly rich in chromatin. The granules stain a lighter tint than the coarse variety; they vary slightly in size in different cells.

III. Lymphocytes are few in number are apt to be mistaken for nucleated red corpuscles which have lost their haemoglobin and whose nuclei have become rounded.

The cytoplasm of the hen's lymphocytes is less basophil than the nucleus.

The total number of non-granular white cells is not above 10% of the whole number of leucocytes.

Action of nucleic acid on the blood and bone marrow

In blood films taken after nucleic acid injection, there is a noticeable difference in the relative numbers of granular to non-granular forms. Instead of the usual 10% or less, there is now at least 40% of non-granular cells present. These non-granular cells are seen to differ in variety and a great part of the increase in their number is made up of cells containing nuclei of the same general size and configuration as those of the fine oxyphil cells.

cells.

The coarse eosinophil cells are found in almost the same proportion as the fine oxyphil. They show a distinct tendency to breaking down and loss of chromatin but at the same time many small eosinophils are seen with deeply stained nuclei and closely crowded spindle-shaped granules.. They are most probably young forms.

The finely granular oxyphil cells are very frequently seen to contain much fewer granules per cell than normal, and in fact all transitions can be traced from cells full of granules to forms absolutely devoid of them.

There must have been some effect here on the red corpuscles but it is inappreciable histologically.

The marrow films do not show any obvious changes, probably due to the fact that the animal was killed so soon after the injections.

Summary of the chief microscopic changes following
nucleic acid injections.

A. Blood.

- (1) Hypo - and hyper - leucocytosis
- (2) Increase in size with diminution of staining power of nuclei of granular leucocytes.
- (3) Diminution in number of fine oxyphil granules per cell.
- (4) Alterations in staining reaction of both coarse and fine oxyphil granules more especially in the rabbit.
- (5) Looser and more fragile structure of granular cells.
- (6) Probable relative increase in number of coarse eosinophils.

B. Marrow.

- (1) Distinct congestion of the marrow of long bones passing into -
- (2) a condition resembling cloudy swelling, in which the nuclei seem exhausted of their chromatin and granules (only examined in rabbits).
- (3) Variations in reaction of the granules to basic and acid dyes, far beyond that seen occasionally in rabbits.
- (4) Great diminution in number of eosinophil cells

cells in marrow.

Lungs.

Distinct congestion with relative increase of
leucocytes.

ADDENDUM.

It would have been interesting to have investigated the effect of nucleic acid administration on the granular leucocytes found in pathological cases, but unfortunately there has been neither opportunity nor time for it.

During last winter (1898-99) two very typical cases of leucocythaemia, one of each variety occurred in the Royal Infirmary but as the patients were both very ill it was of course impossible to attempt any such enquiry. Permission was, however, kindly granted me to examine the urine.

The points I wished to determine ^{were} ~~was~~ whether there was any difference in the P_2O_5 excretion or other bodies derived from nuclein in cases of spleno-medullary as compared to lymphatic leucocythaemia.

In the paper before mentioned, ⁴⁹ Dr Milroy and I had given the results of the quantitative analysis of the excreted products of nuclein in leucocythaemia and had shown in one case of the spleno-medullary variety, a distinct diminution in the phosphorus excreted, pointing very decidedly to a diminished breaking down of nuclein in that disease. On the other hand, it was pointed out that in "nucleic acid"

acid" leucocytosis there was a distinct rise in this excretion amounting to several times that taken in.

I. In the case of lymphatic leucocythaemia the urine was collected carefully at the end of every twenty-four hours and analysed quantitatively for P_2O_5 , Nitrogen, (total), Uric Acid and nuclein bases by the methods already mentioned.

Here the diminution of P_2O_5 was well marked showing the close connection of the two forms of the disease. The daily average taken from ten observations was 1.17 grm. while the total nitrogen averaged 10.28 grm. thus giving a ratio of P_2O_5 to Nitrogen of 1 to 8.7, whereas the usual average is 1 to 4.5 or 5. The number of leucocytes averaged 250,000. The excretion of uric acid and nuclein bases was slightly increased relatively to the total nitrogen excreted.

Thus the uric acid excreted daily averaged .45 grm. while for the same amount of nitrogen I found in a healthy person .35 grm. and in the same way for the nuclein bases in the leukaemia case .009 grm. daily and in a healthy person for the same total nitrogen .008 grm.

This increased excretion of bodies which may

may be artificially derived from nucleins is not necessarily due to their decomposition in the body for even in artificial disintegration as shown in the first part of this paper, the phosphorus-holding part of the nucleic acid is still in organic combination at the point where the alloxur bodies are split off, and hence it is reasonable to suppose that these bodies may be cast off from the nuclein during metabolic changes or may even arise in the cytoplasm of the cell from bodies unconnected with nuclein. (Mares⁵⁰).
(For actual figures see table of analysis of urine).

Urine in Lymphatic Leucocythaemia (Dixon).

	Date	Quantity c.c.	Specific Gravity	Total N ₂ grms.	P ₂ O ₅ grms.	N ₂ of Uric acid.	N ₂ of Nuclein bases.	Leucocytes
	Feb. 9	1047	1017	9.205	.982	.138g	.010g	
	- 10	1065	1019	9.405	1.535	.133	.006	
	- 11	1150	1020	10.976	.977	.157	.007	238,000
	- 13	892	1020	9.291	1.189	.188	.005	
	- 14	1220	1018	10.726	.886	.141	.008	
	- 15	1270	1015	10.596	.569	.241	.009	
	- 17	1050	1018	9.584	1.141	.109	.010	320,000
	- 18	1079	1019	10.211	1.294	.080	.008	
	- 19	1108	1020	10.796	1.426	-	.011	
	- 21	1325	1016	12.020	1.705	.247	.017	300,000
Averages				10.281	1.170	.159	.009	

II. The second case was one of spleno-medullary leucocythaemia and is also interesting from the same point of view.

view.

The excretion of phosphorus here was not so markedly diminished as in the other cases but this is fully accounted for, when we note that the leucocytes were markedly diminishing in number in the blood during the examination. Thus the P_2O_5 excretion during the first four days examined averaged 3.5 grm. while the total nitrogen was 18.6 grm. this gives a ratio of 1 to 5.3, not a very marked alteration, although still one along the same lines; - but this ratio becomes all the more interesting when we note that the numbers fell from about 70,000 per cmm before the analysis was begun to 36,000 just after the fourth day's result.

A week was then allowed to elapse and the urine again analysed for four days. By this time the leucocytes had fallen to 2.000 per cmm. The ratio of P_2O_5 was then 1 to 5.8 (See table).

The amount of alloxur bodies excreted was greater during the first series than the second: - thus the amount of uric acid averaged .75 grm. on the first four days, (for the same amount of nitrogen a healthy person excretes usually .6 grm) while on the second four days the amount was .6 grm - that

that is, an average normal amount. In the same way for the nuclein bases on the first four days .024 gm. during the second four days .018 while the normal excretion would be .014 for the same amount of nitrogen.

These facts point to a diminished leucolysis in leucocythaemias of both kinds - but that when the leucocytes diminish in number in the blood the products of nuclein metabolism may approach the normal amounts.

Urine in Spleno-Medullary Leucocythaemia (Hunter).

Date	Quantity c.c.	Specific Gravity	Total N ₂ grms. 2	P ₂ O ₅ grms.	N ₂ of Uric Acid	N ₂ of Nuclein Bases	Leucocytes
Oct. 11	1389	1018	15.634	3.565	.272	.044	60,000
" 12	1657	1017	20.878	3.468	.342	-	
" 13	1535	1020	19.083	3.397	.190	.013	
" 14	1542	1020	18.824	3.578	.193	.016	
" 15							36,000
Oct. 22	1531	1019	21.262	3.878	.238	.025	
- 23	1217	1021	16.691	2.949	.164	.011	
- 24	1394	1018	18.501	3.079	.250	.019	
- 25	1451	1018	18.445	2.837	.161	.016	2,000
Averages.							
First series.			18.604	3.504	.249	.024	
Second series.			18.724	3.185	.203	.018	

General remarks on the results obtained from my experiments with the conclusions that may be drawn from them.

I must, in the first place, re-capitulate some of the conclusions which I arrived at from the study of the effect on the general metabolism of the nucleins in man, as it was from a study of this subject that I was led to work out the more intricate intracellular changes that take place in the leucocytes present in the blood and marrow, after nucleic acid is given in varying doses.

A. General metabolism of the Nucleins.

(1) There is no doubt that after taking nucleins (or tissues containing these bodies) and nucleic acid, there is a distinct increase in the number of leucocytes in the circulating blood. As soon however as the phosphorus in these bodies passes from an organic to an inorganic form, this action on the white cells is no longer obtained.

(2) With the increase in the number of leucocytes after the action either of nucleins or nucleic acid,

acid, there is a very remarkable increase in the P_2O_5 excretion, both absolute and relative, this rising to a far greater height than could possibly be accounted for from the amount of phosphorus taken in the form of the organic compounds above referred to.

When one remembers also, that from my first published series of experiments, it was seen that all the phosphorus in the nucleic acid compounds was not absorbed, one is forced to the conclusion that there must be within the body some intracellular change that has resulted in this marked increase.

When the phosphorus was given in an inorganic form, even in one closely allied to nucleic acid, viz., mono-metaphosphoric acid, this increase was not seen at all, or rather any slight rise was inappreciable compared to the large amount of the inorganic acid taken by mouth.

Therefore the agent which causes the leucocytosis, causes the increased P_2O_5 excretion, while the one that fails to produce the former also has no effect on the excretion of the latter.

(3) Goldscheider and Jakob and succeeding authors have taken up mainly, if not entirely, the numerical

numerical alterations in the leucocytes after the action of different animal extracts, but I was led from my previous work on the subject to expect that more than simply altered distribution due to chemotaxis lay at the root of the matter and so commenced the investigation described in the preceding pages, - since it seemed so probable that the increased excretion of phosphorus was due to an altered metabolism of the cells in the blood, or marrow, or both.

I shall now^w state my conclusions as to the characters of the granular cells in blood and marrow of the different animals examined, and then the effect of nucleic acid on them.

As emphasised in the preceding pages it is the granular cells which are of special importance in this respect.

B. Characters of the granules in the two great types of granular cells.

I. Effect of Reagents.

- (a) alcohol - (boiling) - no solvent effect on either type.
- (b) ether - also boiling - no solvent action.
- (c) alkalies - in watery and alcoholic solutions (including sodium ethylate) removes the great

great majority of the amphophilous granules while the great bulk of the coarse eosinophilous are unremoved but altered in reaction so that they stain more deeply in eosin.

- (d) Acids - used in the same media produce same results but accentuate the greater resistance of the eosinophilous compared to the amphophilous.
-

From the above, one can exclude the view that either of those two forms of granule contain fat or lecithin and they are decidedly organic in type, so one is led to believe that they are albuminous.

From their staining reaction, especially as we have seen after the action of nucleic acid, it seems almost certain that both are combinations between nuclein and varying amounts of albumin.

In the amphophilous there is undoubtedly a larger amount of the element which has an affinity for basic stains than is present in the eosinophilous: but the combination between the two constituents is looser in the former than in the latter.

The reasons for these statements are, that the amphophilous stain with both acid and basic dyes, while the coarsely granular only takes up the acid

acid ones.

This proves that the stains in the former case can more easily enter the molecule and fix either the basic or acid constituent, while in the latter it is the molecule as a whole and not its constituent parts which react with specific dyes.

As we have already seen, nucleic acid brings out the difference in this respect in a most striking manner.

II. Morphological distinctions between the two great types of granular cells in different animals.

Here I shall collect the results which I have obtained from the examination of the granules in the cells of the blood and marrow of control animals.

- (a) In Rabbits - In the rabbit, the amphophilous granules are small refractile bodies filling the whole cytoplasm as a rule. They are truly amphophilous especially in simply dried films. On the other hand, the eosinophilous granules are larger, more regular in contour, less refractile, and take on more of a dull red tint in eosin.

The cells bearing the latter form of granule

granule are very much less numerous

- (b) In Dogs. - There is here a marked difference between the two forms. The fine oxyphil granules are so fine, so closely packed and refractile that the cytoplasm appears homogeneous in most stains. In triacid mixture the granules are fairly clear but still not so well defined as in rabbits and guinea-pigs.

The eosinophilous granules are much larger and except for their fewness in number would be very easily demonstrated. They stain in triacid of a purple tint and are uniform in size.

- (c) In Guinea-pigs. The fine oxyphil granules approach in general characters to the coarse variety but there is a marked difference in the depth of the eosin tint in the two - which is best seen in the marrow. The true eosinophils being of a deep, almost carmine tint while the fine oxyphil ones are brighter, smaller, and more refractile.

- (d) In Hens. The two forms are very distinct here.

here. The fine oxyphil granules are always rounded and vary somewhat in size in different cells. The coarse oxyphil ones are typically spindle-shaped but stages in the development of this, from a simply rounded form can be seen in the same cell to some extent, but always distinctly on examination through a series of cells. The fully developed granule is a long rod-like body pointed at each end, and often possessing a central round vacuole seen distinctly on focussing. These rods lie side by side so that when a cell breaks down they are often arranged in a whorl round the nucleus. They stain very deeply in acid dyes and the number of cells containing them approaches that of the other variety.

C. Effect of Nucleic Acid upon the granules in the cells in blood and marrow of animals examined.

(A) Rabbits

- (1) There is a marked alteration in their distribution. The numbers found in the lung capillaries are increased: in the blood there is first a fall and then a rise; and in the marrow there

there is a diminution of the coarse oxyphil forms.

- (2) The number of granules per cell is diminished first of all in the amphophilous and later to some extent in the eosinophilous.
- (3) The staining reactions of the granules become altered. This change consists in a gradual disappearance of the pure oxyphil reaction first of all affecting only the amphophilous and even in those simply isolated granules without affecting the whole number in each cell.

This is of very great interest because with the gradual disappearance of the oxyphil reaction a slowly increasing basophil tendency shows itself.

The same holds good of the coarsely granular. I believe that this is undoubtedly due to the fact that the albumin which is loosely bound to the nuclein in the amphophilous granule is first of all removed and hence the more acid residue gradually takes up more and more of the basic element in the stain. This, as already described, is more marked in the amphophilous for the reason that the combination there is not nearly so firm

firm as in the true eosinophilous. It is only later that the nuclein of the residue begins to leave the cytoplasm and at this stage, the granules in each cell are much fewer in number.

With regard to the true eosinophilous, this basophil reaction also occurs at a later period after the action of the acid. One must remember however that these granules are more acid than the amphophilous in the original condition; but the compound between the nuclein and the albumin is a firmer one, although the amount of the latter is larger. Such a condition also occurs in the vitellin granules in carps' eggs. The decrease in number of the granules per cell after the action of nucleic acid is not so marked as is the case with the amphophilous, or rather it is more difficult to find cells in the intermediate stages before complete loss of the granules. This latter condition seems undoubtedly to occur after prolonged action of the nucleic acid.

Again a point of great importance is that in the stage of recovery after nucleic acid, there is a gradual return in the case of both amphophilous and eosinophilous to the original

original condition both as regards reaction, number of granule per cell and number of granular cells in marrow.

- (4) The changes in the nucleus are also distinct. There is an increase in size with loss of the chromatin, but changes in such a composite body cannot be so easily observed as in the well defined granules.

Dogs.

- (1) There is again that curious distribution in blood and lung capillaries explained on Goldscheider and Jakob's theory of chemotaxis.
- (2) As already stated, it is almost impossible to make out the alterations in reaction and number of granules referred to in rabbits, for the reason already fully given.
- (3) The changes in the nucleus are of the very greatest importance because in this case I was able to make out what is probably the first stage in exhaustion of the nucleus, viz., the increase in the size of the zone which surrounds the central core. This peripheral zone takes on the basic stain to a much less extent than the central

central part - a change which must be due to a gradual removal of the nuclein - probably going to supply the cytoplasm.

C. Guinea-pigs.

- (1) Distribution is affected here as in the other cases.
- (2) Indications of the same change in reaction are seen but the combination between the nuclein and albumin in both types of oxyphil granules is a firmer one than in the case of rabbits. One must remember however that in this case smaller quantities of nucleic acid were given and ^{marrow was} examined only after a short time had elapsed since the acid was injected.
- (3) Distinct changes in the nuclei of granular cells of both kinds are seen. These are of the same nature as in the case of the other animals.

D. Hens.

These were not examined along the same lines, but the effect on the granular cells of the blood ~~was~~ ^{is} of the same nature as in the case of rabbits. That is to say the fine oxyphil granules ~~are~~ ^{were}

~~are~~ the first to be affected - their granules becoming discharged and their nuclei exhausted.

The same changes are seen to be occurring in the coarse granules and here these have been apparently stimulated to proliferation.

General Conclusions.

- (1) In a paper already published there was shown to be a decided rise in the excretion of phosphorus as a sequence of the ingestion of nucleic acid.
- (2) In animals this is here shown to be accompanied by a hypo- and hyper-leucocytosis in which the granular cells are the forms affected and in which a heaping up of leucocytes in the lungs takes place.
- (3) Following the injection of nucleate of soda into animals, especially rabbits, there are microscopic changes in the granular leucocytes of the following kind:-
 - (a) Swelling of the nucleus with deficiency in staining power- resembling to some extent the changes seen in secreting gland cells.
 - (b) Marked increase in the number of cases of cells showing granules with a tendency to take on the basic stain- pointing to an unstable equilibrium of the molecules and hence increased metabolic activity.
 - (c) Diminution in number of granules in some and even their disappearance, pointing to exhaustion of the cell.
- (4) At a later period, the bone marrow is affected:

affected: it shows:-

- (a) Distinct congestion
 - (b) Increase in parenchyma at the expense of the fat cells.
 - (c) Changes in the granular marrow cells of the same nature as in the blood.
 - (d) These proceed to a stage of exhaustion where the appearances resemble cloudy swelling and in which the coarse oxyphil cells almost disappear.
 - (e) Recovery from this condition begins about 75 hours after the injections.
- (5) Experiments on the chemical nature of the oxyphil granules point strongly to their being of the nature of nucleo-proteid, ~~and~~ (hence, phosphorus-holding); but that they are of two distinct non-interchangeable classes - fine and coarse - a difference also accentuated by other points.
- (6) The co-relation of increased excretion of $P_{25}O_5$ with the histological and micro-chemical changes in the granules and nuclei of oxyphilous cells points to the close relationship between these two structures, and the probable origin of the former from the latter.
- (7) The granules in blood and marrow cells after

after nucleic acid injection are not different in their solubilities in acids and alkalies from those found normally.

(8) Investigation of the metabolism of nuclein in a case of lymphatic leucocythæmia shows that the alterations are in the same direction as shown before in a case of spleno medullary, i.e., there is diminished phosphorus excretion, pointing to diminished nuclear katabolism.

(9) In a case of spleno-medullary leucocythæmia where the leucocytes diminished markedly during the investigation there was a partial return of the nitrogen to phosphorus ratio to its normal figure.

This work has been done in the Physiological Laboratory of the University of Edinburgh.

List of Specimens sent in.

Rabbit I. Normal appearances.

- (1) Marrow Film, Rabbit I. showing normal appearances of cells.

Fixed in formalin vapour, stained with methylene-blue-eosin (Chenzinsky).

- (2) Blood film Rabbit I. showing normal appearances of cells in the blood.

- (3) Marrow Section Rabbit I. cut in paraffin - stained 18 hours in Chenzinsky's methylene-blue-eosin.

- (4) Marrow Section Rabbit I. same stained with eosin and Löffler's methylene Blue.

Rabbit II.

Killed 18 hours after injection of nucleic acid.

- (5) Marrow film stained with eosin-methylene-Blue.
(6) Blood film fixed and stained as above.
(7) Marrow Section stained with Chenzinsky's eosin-methylene-blue.
(8) Marrow Section stained with Löffler's methylene blue and eosin.

Rabbit III.

- (9) Marrow film fixed and stained as before.

- (10) Blood ditto
- (11) Marrow Section (Chenzinsky)
- (12) ditto (Löffler).

Rabbit IV.

- (13) Marrow film as above.
- (14) Blood film.
- (15) Marrow Section Chenzinsky.
- (16) " " (Löffler).

Dogs.

- A. (17) Blood film taken before injection of nucleic acid. Fixed by heat and stained in triacid.
- (18) Blood film after injection of nucleic acid, treated as above.
- (19) Marrow film - fixed by heat and stained with triacid.
- (20) Marrow of control dog (B) for comparison - treated in same way.

Guinea-Pigs.

- (21) Control guinea-pig marrow film heat - eosin and Haematoxylin.
- (22) Guinea pig "C" marrow film showing effect of nucleic acid.
- (23) Control guinea pig marrow film stained with Chenzinsky (16 hours).

- (24) Guinea-pig "G", marrow film showing effect of nucleic acid to be compared with (23) (fixation and staining same in both).
- (25) Guinea-pig "G" lung showing accumulation of leucocytes in the capillaries.

Hens.

- (26) Hen's blood film - before nucleic acid.
- (27) Hen's blood film after nucleic acid injection (18 hours). Both films fixed with heat for same time and stained in Huber's mixture.

Solubility of Oxyphil Granules.

- (28) Guinea-pig marrow film fixed with heat and stained with eosin and methylene blue for comparison to the others.
- (29) Effect of oxalic acid (compare ^{with} ~~to~~ 28).
- (30) Effect of Acetic Acid in Alcohol.
- (31) Effect of Alcohol and ether.
- (32) Effect of sodium ethylate.

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Plate I Description of Figures.

PLATE I

Rabbit I. Marrow film fixed with formalin
vapour, stained in eosin and methylene blue

(Chenzinsky) ($\frac{1}{12}$ oil. immers. Oc. 8)
~~+12~~

The amphophilous cells are seen with well defined
outlines and fairly well stained nuclei.

Two eosinophilous myelocytes are seen, the one on the left containing some basophil granules
Two erythroblasts with pink granules in its nucleus are
seen and also a finely granular basophilous cell.

PLATE II.

Rabbit I. Blood film fixed and stained as above.

- same magnification.

Several amphophilous cells are seen and one eosinophil
in the upper part of the field. The cells are
crowded with their granules and ^{the} nuclei ^{are} distinct.

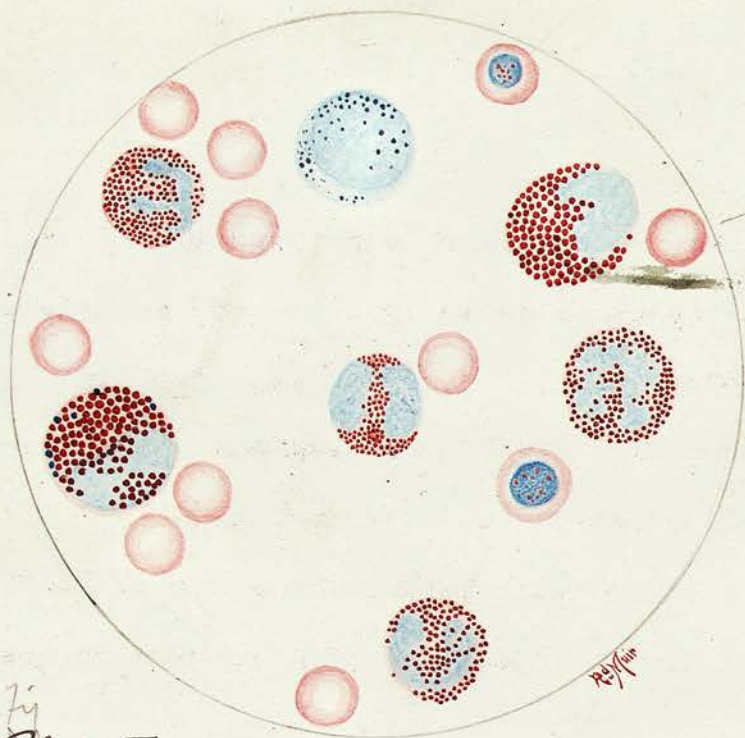


Fig
Plate I

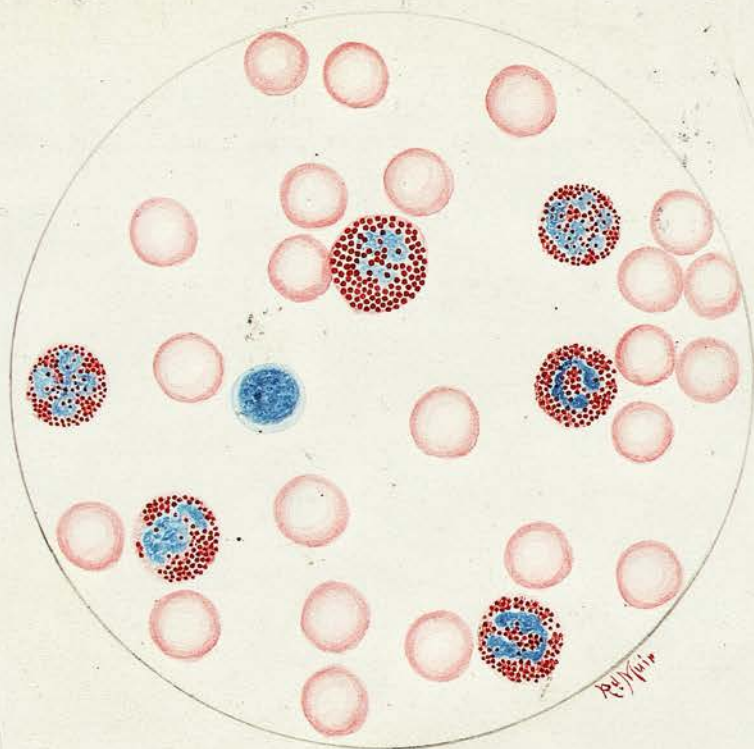


Plate II

PLATE III.

Rabbit II Marrow film fixed with formalin and
(Chenzinsky)
stained in eosin and methylene blue ($\frac{1}{12}$ ol.

immers. Oc.8) (This shows the effect on the
granular cells at eighteen hours after the injection
of nucleic acid)

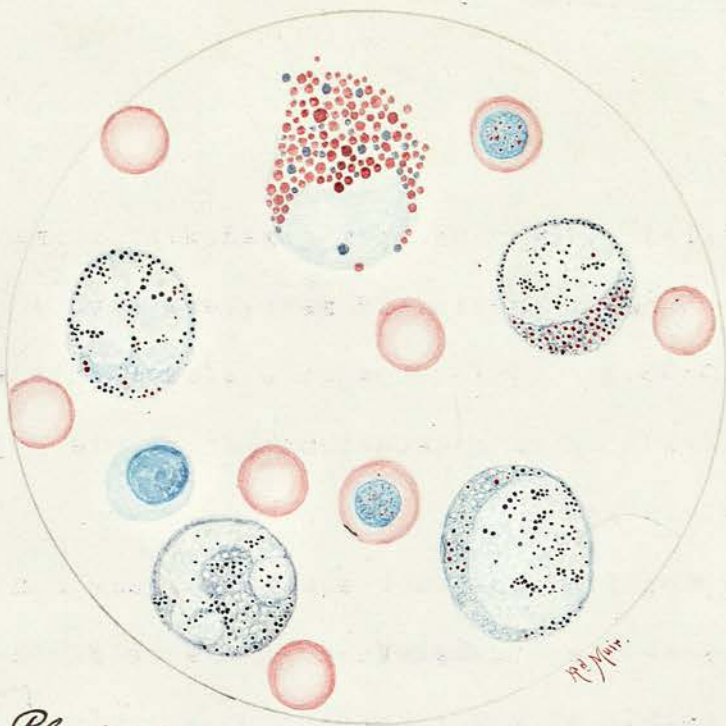
Three amphophilous cells are seen larger than normal,
with pale swollen nuclei and granules which have lost
their normal acidophil reaction to a great extent and
stain in the basic dye. *The granules have also been
partly discharged.*
A large, diffuse eosinophil cell seen with granules
altered in reaction.

PLATE IV

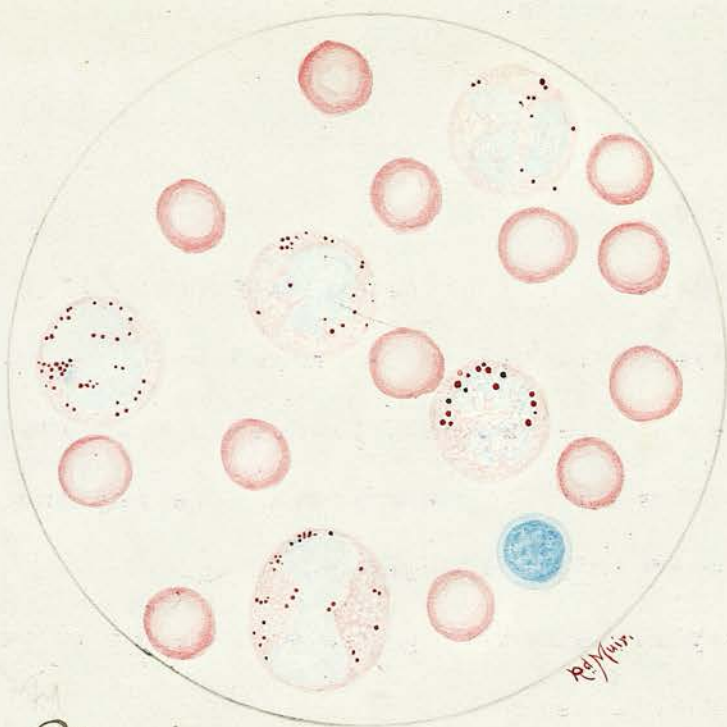
Changes in blood cells of Rabbits II & III
fixed, stained and examined as above.

The nuclei of the amphophilous cells are swollen and
poor in chromatin. The granules are few and stain
in both acid and basic dyes.

Unstained areas seen corresponding in position to granules
An eosinophil cell with granules altered in reaction
is seen to the left. *Right.*



Plati III



Plati IV

PLATE V.

Rabbit III. ^Marrow film fixed with formalin
and stained in eosin and methylene blue (*Cheninsky*)
($\frac{1}{12}$ ol. immers. ~~Oct. 8~~ Oc. 8) (To show effect on marrow
at 50 hours after injections ceased.)

Amphophilous cells are seen with very pale nuclei,
loosely arranged granules which are altered in reaction.
The eosinophil cells ^{is} ~~are~~ most markedly affected at
this stage as seen in the loss of acidophil tendency
in the granules, loose structure of the cell, and
paleness of nucleus.

PLATE VI.

Rabbit IV. ^Marrow film fixed, stained and
examined as above. (To show the appearances
seen in recovering cells. Animal killed at 75 hours.)
The normal reaction of the granules has to a great
extent returned in the case of the amphophilous
whilst the eosinophilous are still deeply affected.

(~~are~~ slightly more affected
than shown in the plate)

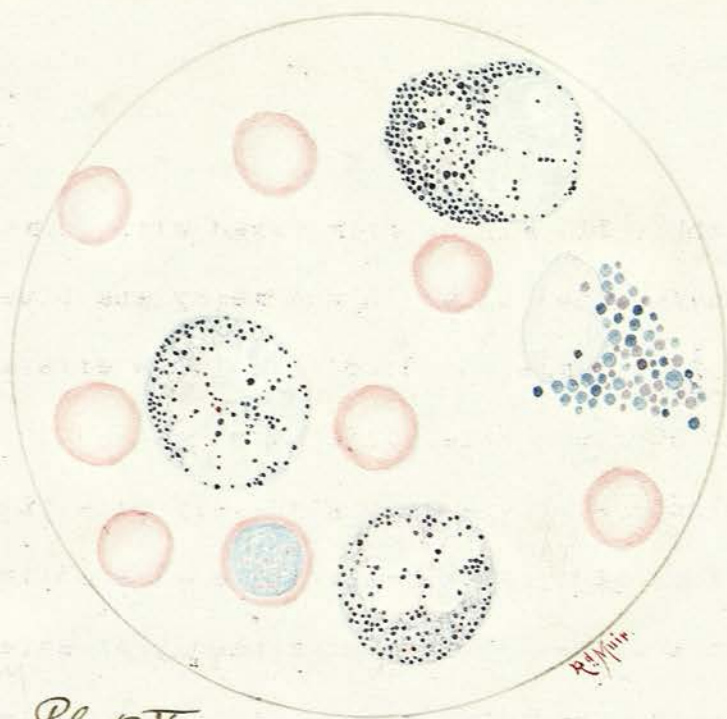


Plate V

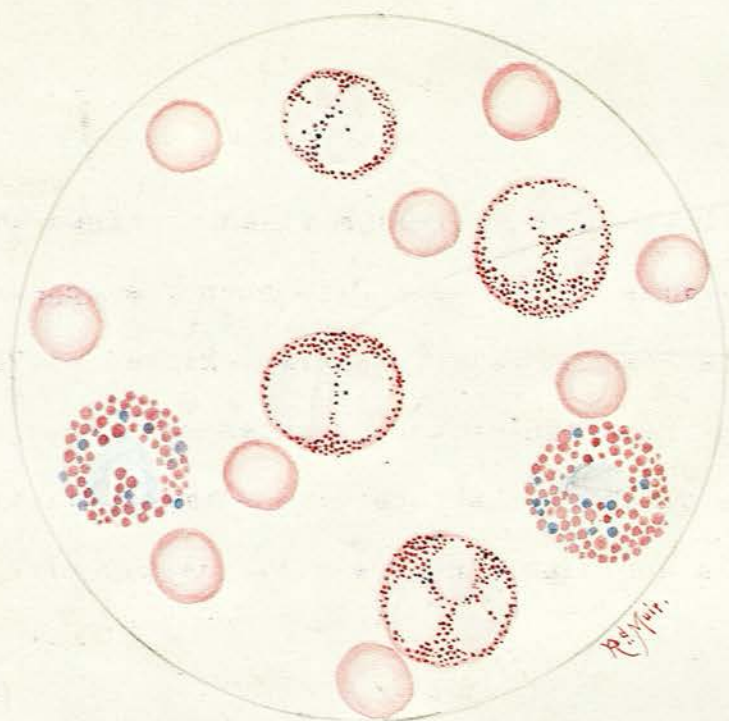


Plate VI